PROCEEDINGS

TECHNOLOGY TRANSFER CONFERENCE No.4

PART 2

AIR POLLUTION RESEARCH

CONSTELLATION HOTEL , TORONTO

NOVEMBER 29&30 , 1983

SPONSORED BY

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RESEARCH COORDINATION OFFICE

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MINISTRY OF THE ENVIRONMENT

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November 29 and 30, 1983

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AIR POLLUTION RESEARCH

Towards an Electrochemically-Based Chemoreceptor
For Trace Atmospheric Organics

by

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ABSTRACT

Natural chemoreception provides a model for development of selective organic sensing where the basic premise of operation implies that transduction of a selective binding event occurs via change of electrochemical conductance features across sensing membranes. This work describes the construction and theory of an ammonia gas sensor based on an artificial bilayer lipid membrane (BLM) which was modified for selectivity with the antibiotic nonactin. Detection limits are presently in the 10^{-6} M range and selectivity coefficients for ammonia over methylamine and other similar organic species are extremely high. Presently limitations of sensitivity and selectivity are determined by the chemistry of the BLM. This has been investigated with respect to transmembrane ion conduction energy barriers and the alteration of the inherent membrane dipolar potential for development of a more generalized mechanism for signal transduction through employment of selective receptors. Preliminary results of Langmuir-Blodgett thin-film deposition technology for the production of stable organized membranes suitable for routine laboratory sensing will also be introduced.

INTRODUCTION

A significant majority of analytical measurements made today are concerned with the qualitative and quantitative analyses of organic species in complex matrices. The most common route for such analyses involves complete separation of sample components followed by identification and quantitation. However this process becomes increasingly difficult for complex samples and is unjustifiably expensive on both a temporal and financial basis if information is required for only a few components in the sample. This work attempts to investigate and evaluate the potential of an electrochemically-based detection system simulating that used by natural living systems for chemoreception with respect to selective organic sensing.

Advances of selective sensors have evolved dramatically over the last 15 years in the field of inorganic analysis. These sensors are based on the ion-selective electrode, the best known being the glass electrode which was initially developed in the 1920's. Analogous development of selective electrodes for organic compounds has also occurred over the last 10 years with varying degrees of success. Generally these systems are best described as hybrid electrodes, incorporating an inorganic ion-selective electrode measurement system with an organic selective membrane capable of generating the appropriate inorganic ion. The ion-selective electrodes encounter interference problems from ions of similar character, solubility dissociation or dissolution of sensing membranes and general ionic strength-ion activity relationships. The resulting potential is of mixed origin and generally limits detection of inorganic species to concentration ranges greater than 10^{-6} M. Organic selective electrodes have comparable detection limits since final measurement is performed by the inorganic ion-selective device. The interesting feature of the organic selective electrodes stems from the employment of complementary reactive enzyme-substrate or antibody-antigen pairs which establish selectivity. This has also been used to great advantage in immunoassay technology which represents another technique where selectivity is determined on the basis of reaction chemistry. However this system again requires separation techniques and is unsuitable for direct concentration determination. Considering this disadvantage and the poor detection limits of potentiometric electrodes, a distinct lack of a selective and sensitive electrochemical method for trace organic analysis without involvement of separation becomes obvious. Such technology is apparently employed by the chemoreceptive sensory systems of living organisms.

Interest in basic biochemistry has stimulated investigations of the chemoreceptive funnctions of natural living organisms. An overview of the mechanisms proposed for processes such as olfaction indicate that the electrogenic activity implicated in chemoreception at certain cell membranes provides an interesting model for the development of electrochemically baseed sensors(1). Such a direction is offered by the highly sensitive and selective

interactions that occur between particular biological macromolecules, receptors and their complement ligands resulting in electrochemical, physiological and biochemical action at cellular localities. In order to study interactions at or across cell membranes, biochemists required a well characterized, easily prepared synthetic model resembling the structure and functions of natural systems. Such a structure, consisting of a planar bilayer of lipids was reported by Mueller et al.(2) in 1962 and has been termed the bilayer lipid membrane (BLM). These membranes of 6-8 nm thickness consist of two adjacent lipid monolayers oriented in a symmetrical but opposing manner as illustrated in Fig. 1. Chemically this structure has a central non-polar hydrocarbon region bounded on both sides with polar sheets of lipid headgroups which are hydrated in supporting aqueous electrolyte. A direct electrical potential applied across this structure will result in the passage of a small but finite ion current through the membrane. A typical current-voltage curve for a stable BLM has ohmic response over a range of approximately -60 to +60 mV with the current usually being on the order of 10^{-9} A/cm^2. Incorporation and membrane interaction with molecules or complexes which alter the standing transmembrane ion flux results in the production of an analytical signal. We have suggested that a membrane-bound receptor/stimulant interaction which could translate the binding event into a transmembrane ionic flux is a suitable model for the development of a sensitive and selective electrochemical sensor(1,3). A substantial number of substances will induce conductance changes in BLM. These include non-selective interactions with antibiotics and simple organic molecules as well as selective interactions of enzyme-substrate, antibody-antigen, hormone-receptor and olfactory receptor systems.

Since the basic premise for analytical signal generation is that a membrane embedded molecular compound or complex can in some way alter lipid matrix properties to influence transmembrane ion flux, it becomes important to establish which physical features of the membrane are responsible for limiting the ion current. Ion flux is governed by features such as membrane-external ion concentration, applied potentials and membrane internal fluidity, thickness, dielectric constant, surface charge and surface dipolar potential. Since typical BLM forming lipids contain a polar region with elements of differing electronegativity, it is anticipated that a net molecular dipole moment may exist. When lipid molecules are organized and packed into the BLM structure, such net dipoles concurrently become ordered and combine to create a plane of ordered dipolar charge. By virtue of this salient feature of a BLM, a net positive potential is generated and superimposed across membranes formed from neutral lipids(4,5). This electrostatic field is directly responsible for control of transmembrane ion flux and represents a static situation which can be perturbed to induce analytical signal generation. Two general mechanisms involving selective interactions are suitable for BLM stimulation eliciting an analytical response. These rely on either the creation of an ion conductive pathway within the membrane or on surface dipole or

charge alteration, reflecting changes in either the chemical or electrical potential energy barriers respectively.

Application to Gas Sensing

Ideally a BLM gas sensing device will operate on the basis of selective interaction of a membrane embedded receptor with the small volatile organic of interest, resulting in a concentration dependent increase in conductance via one of the aforementioned mechanisms. The lack of knowledge in the area of receptor implementation and the present lack of suitable receptors restricts the employment of such a general mechanism. However specialized systems which demonstrate the usefulness of the lipid membrane matrix as a basis for receptor design can still be established.

Numerous conventional selective gas sensing electrodes are available for small molecules such as carbon dioxide, sulphur dioxide, nitrogen oxides, hydrogen sulphide and ammonia. All of these sensors rely on the same mechanism for signal generation, employing an optional external selective polymer membrane to control gas permeation into a thin film aqueous phase where solution equilibria cause substantial pH alterations which are readily measured with a glass electrode. Limitations for these systems often originate with solvent or electrolyte contamination problems, or are controlled by other competitive features involving the ion-selective elctrode or semi-permeable membrane. The BLM sensor does not succumb to the competitive effects which limit the use of ion-selective electrodes since the development of an equilibrium interfacial potential is not the primary source of analytical signal. In order to demonstrate the feasibility of gas detection with a BLM, a working prototype designed to measure ammonia gas has been developed. The general electrochemical and membrane support design characteristics are similar to those employed for a conventional glass electrode based ammonia sensor, but the glass sensing membrane normally employed for interfacial potential development is replaced by an ammonium conductive BLM.

This report will summarize the operation of the prototype BLM gas sensor and will describe the chemistry of the BLM from the standpoint of ion conduction for future implementation of the general receptor mechanism for selective detection.

INSTRUMENTATION AND EQUIPMENT

Electrochemistry

The electrochemical cell consisted of two identical machined perspex blocks separated by a teflon sheet (0.1 mm thickness) which contained a circular aperture (1 mm diameter) used for BLM support as illustrated in Fig. 2. An external direct potential was applied across the membrane between two agar salt bridge extended Ag/AgCl single junction reference electrodes (Orion Research Inc., Cambridge, Mass). The external circuitry consisted of a DC power supply and a microprocessor controlled multichannel digital electrometer (Keithley System 1, Keithley Instruments, Cleveland, OH) for data acquisition. An optical system consisting of a cold light halogen-quartz fiber optics illuminator and a wide angle twenty power microscope were used to investigate and monitor BLM formation for electrochemical studies. The solution cell and sensitive electronic equipment were isolated in a well grounded Faraday cage.

Capillary Gas Chromatography

A conventional packed column Varian Aerograph 2740-10 Gas Chromatograph (Varian Associates, Walnut Creek, CA) was modified for capillary gas chromatographic investigation of lipid and cholesterol oxidation. A glass lined splitter assembly, Inlet Splitter System (Chromalytic Technology P/L) including a septum purge and buffer volume to reduce gas viscosity effects replaced the conventional inlet. Stainless steel glass lined inlet and nickel outlet tubing was of 0.25 mm internal diameter and included a make-up gas tee junction at the end of the column to reduce dead volume effects. The detector was based on a sensitive flame ionization electrometer assembly employing hydrogen/air fuel mixtures and helium carrier and make-up gas. A separate gas flow controller (Pressure Control System, Chromalytic Technology) was used due to the low volumes of gas required. The capillary column employed for this work was a 30 m x 0.25 mm ID, Durabond fused silica column with DB-1 (equivalent to SE-30) 0.25 μm coating and a practical column efficiency of N_{eff} = 100,000 (J + W Scientific, Inc., Rancho Cordova, CA).

Gas Sensor

The basic design of the gas sensor electrochemical cell retained the concept of two solution compartments which were separated by a BLM. A BLM was formed in a standard teflon sheet, and was supported on one face by a conventional perspex half cell. A porous teflon semi-permeable membrane designed for use with an ammonia electrode (Ammonia Porous Membranes, Orion

Research Inc.) was used to trap a thin layer of electrolyte at the other membrane face and allowed interfacing to the gas phase. The trapped aqueous layer was connected by means of a thin channel to a Ag/AgCl reference electrode as seen in Fig. 3. A specially constructed external clamp was used to sandwich the teflon sheet and semi-permeable membrane to a central perspex sheet, allowing for easy apparatus cleaning and teflon replacement. External electrochemical equipment was oraganized as previously described.

PROCEDURES

Formation of BLM

The BLM were formed from solutions containing n-decane (which had been purified over an alumina column and dried over molecular sieves) and lipid/cholesterol in a 2%/2% weight-to-volume ratio. The lipid containing solution was first placed in an ultrasound bath for 1 minute to insure homogeneity and was then introduced to the 1 mm diameter teflon aperture by means of a fine sable hair brush. The resulting lipid plug trapped in the aperture between the two 5 ml aqueous solution compartments containing aqueous electrolyte would spontaneously thin to form a region consisting of a BLM, surrounded by a torus of excess lipid and solvent. (All experiments in this work employed 0.1 M KCl electrolyte and an applied potential of +25 mV unless otherwise stated). Observation of BLM formation could be directly accomplished through use of electrical monitoring of transmembrane charge passage or by surface optical reflectivity properties (6). Experiments requiring elevated temperatures were initiated as those described at room temperature. Stable BLM were prepared at $21 \pm 1°C$ and then an infrared heating lamp was employed to increase the temperature at a rate of approximately $1°C$ /min.

Sample Preparation for Chromatography

Phospholipid modification for TLC and gas chromatographic analysis consisted of transmethylation to produce methyl esters of the lipid acyl chains. Numerous methods to accomplish this conversion have been described in the literature, though the best method for complete concentration independent conversion of small samples employs a base catalyzed reaction (7). Lipid samples of mass 1-10 mg were reacted for 20 minutes at 22°C in a reagent mixture consisting of 1 ml of freshly prepared 0.5 M sodium methoxide and 1 ml of dry benzene. Samples were then extracted with 3 ml of diethyl ether and washed with 3 ml of 1 M aqueous sodium chloride twice. The remaining diethyl ether/benzene

extract was then dried over anhydrous sodium sulphate, concentrated with a nitrogen gas stream and was submitted to the gas chromatograph. Lipid/sterol decane solutions (suitable for BLM preparation) of 50-100 μl volume were similarly prepared without component preseparation for chromatographic analysis. Optimum column operating conditions are provided with the chromatogram shown in Fig. 10.

Gas Sensing Device

The operation of the gas sensor was based on an ion-carrying selectivity for ammonium ion of the antibiotic nonactin, coupled with the action of an ammonia gas semi-permeable membrane. Various electrolyte solutions based on KCl, LiCL and $MgCl_2$ were investigated for effectiveness in enhancing the BLM transmembrane ion current in the presence of standard aqueous concentrations of ammonium chloride at pH $\sim$ 4.0 in the presence of nonactin. Nonactin was added to one solution compartment whille the ammonium solution was introduced in the alternate aqueous phase. After determining the conditions for maximizing sensitivity to ammonium ion detection, one solution compartment was replaced with the gas phase interface allowing ammonia gas sampling.

RESULTS AND DISCUSSION

A BLM-Based Ammonia Gas Selective Detector

In order to demonstrate the feasibility of gas detection with a BLM, a working prototype designed to measure ammonia gas has been developed. The general electrochemical cell and membrane support design characteristics are illustrated in Fig. 3 and are similar to those employed for a conventional glass electrode based gas sensor. The operation of the system depends on a ammonia gas semi-permeable teflon membrane for the purpose of interfacing the fragile free BLM to the gas sample. This membrane offers a pre-selection of the analyte gas, however BLM selectivity to ammonia is maintained by incorporating polypeptides within thhe BLM which preferentially complex and carry ammonium ions rather than electrolyte cations.

Polypeptides which are recognized or classified as antibiotics demonstrate a remarkable ability to distinguish between chemically similar ions and selectively complex such ions for transport across BLM. Of the common antibiotic groups, only the valinomycins and the polyethers are very selective to Group I cations and also ammonium ion. The selectivity is a function of

permeability constants. A summary of such constants is presented
in Table 1 and contrasts the extreme selectivity differences
between gramicidin, valinomycin, nonactin and a polyether. To
reduce the background current measured for the gas sensor and to
maximize selectivity towards ammonium ion, a polypeptide from the
macrotetralide group such as nonactin sould be given preference.
It should be incorporated and operated in a BLM supported by low
concentrations of an electrolyte such as LiCl which is associated
with extremely low residual current to minimize background
conductance and maximize the competitive advantage for antibiotic
binding of ammonium cation. Note that the concentration of
antibiotic also determines the overall analytical signal.
Maximum effect is attained when the antibiotic has a great
selectivity for NH_4^+ over supporting electrolyte cation, and when
the latter is maintained at the minimum concentration required
for stable BLM formation. An ammonium ion concentration-response
curve for nonactin in BLM bathed with $10^{-3}M$ and $10^{-4}M$ LiCl is
shown in Fig. 4. As expected from the difference in sellectivity
of thesee cations, $K_{NH_4}+/K_{Li}+ \sim 10^4$, the limit-of-detection for
this system is $10^{-6}M$ and compares favourably to the commercial
ammonia electrode. Any positive deviation from a linear response
curve may be due to the change in ionic strength associated with
high concentrations of NH_4Cl. Greater selectivity may be
attainable for the purpose of creation of more sensitive ammonium
BLM sensors by appropriate structural synthesis and employment of
crown ethers.

The application of the nonactin/LiCl system in the
configuration of Fig. 3 to detect ammonia gas in a bulk gas phase
has produced the results shown in Fig. 5. The results are time
dependent as expected for a diffusion controlled gas permeation
which results in the production of an ammonium ion population.

Model of BLM Gas Sensor Operation

In order to evaluate the influence of various design
parameters on the described BLM gas sensor, a mathematical model
considering ammonia gas and ammonium ion diffusion through the
experimental system has been developed. The model which was used
for these calculations is summarized in Fig. 6 and illustrates
the various diffusion zones. For simplicity, the area chosen for
the calculations was determined by the area of the BLM used in
this work. This implies that the calculated current data
represents a minimum analytical signal since lateral diffusion
characteristics are not considered. Experimental data is
developed in Fig. 7 to illustrate the trend of observed ion
current as a function of the interfacial concentration of
ammonium ion. Note that the efficiency of the ion transport
process apparently increases as a function of the available
interfacial ammonium ion concentration. This may be due to a
concerted effect caused by reduction of membrane fluidity due to
the order perturbing influence of nonactin, which is concurrently

associated with an ion transport kinetic rate increase. An
analysis of the influence of the thickness of the teflon membrane
and underlying aqueous thin film indicates that the aqueous phase
is the diffusion rate-determining zone and controls the final BLM
ion current.

Analytical Potential

The sensitivity of the modified BLM towards ammonium ion
compares favourably with the commercial form of the ammonia gas
sensor. Treatment of nonactin loaded membranes with up to 0.005M
methylamine hydrochloride did not produce any significant
analytical signal, nor did it affect subsequent analysis of
ammonium ion concentration, indicating a substantial selectivity
advantage for the BLM based sensor. This is necessarily the case
due to the origin of selectivity in these membranes. Since
nonactin complexes the cation in the center of a large
pseudospherical structure, and the cavity at the interior of this
structure determines binding capability (6), it would be
physically unfavourable to complex methylammonium ion compared to
the smaller Group I and ammonium ions. Since methylamine is the
most potent common interferent of the conventional ammonia gas
sensor, and also is physically the smallest interferent, it would
seem that the BLM sensor is effectively immune to interference
from volatile amines. The selectivity of the modified BLM
ammonia gas sensor therefore indicates a substantial
technological advantage over conventional sensors. The solution
experiments indicate that 99% of the final signal can be obtained
for decade changes in ammonium ion concentration over a period of
1 to 2 minutes, implying that these electrodes are adequate for
routine laboratory use when considering time criteria (8).
Calculations indicate that the same holds true for gas phase
sensing with BLM.

This prototype BLM-based gas sensing system illustrates
technical deficiencies with design and function:

- the large volume of trapped aqueous electrolyte must be
reduced to minimize response time and maximize analyte ion
concentration

- transmembrane pressure transients and osmotic pressure
differences must be controlled or eliminated

- a more efficient method of coupling the external electrode
must be devised

- the influence of ion complexing agent in the trapped
solution compartment must be evaluated

- the electrochemical response should be measured on the
basis of integration of transmembrane charge passage since
an equilibrium of partial gas pressure with bulk sample

solution cannot be attained due to the dynamic ion
transport system invoked for this work

   - the semi-permeable teflon membrane used for BLM protection
and work in aqueous solution should be removed when
analyzing in the gas phase

   - the BLM must be supported or altered in some manner so
that it can achieve greater mechanical stability and still
retain its electrochemical sensitivity.

However, these deficiencies do not preclude the further
development of useful BLM-based selective gas sensors. These
systems will eventually offer access to a previously unexplored
realm of analysis when receptors suitable for binding of more
complex gaseous organic species become available.

LIPID MEMBRANE DIPOLE PERTURBATION FOR SELECTIVE SENSING

Origins of Membrane Surface Dipole Potential

   The origins of the dipolar potential can be traced directly
to the intrinsic dipoles found in the lipid membrane polar
regions. This work deals entirely with the lipid phosphatidyl
choline (PC) and, therefore, with dipoles originating from
carbonyl and Phosphorous-Nitrogen (P-N) species, though similar
explanations can be applied for most lipids. A substantial body
of information regarding the orientation of these dipoles in
synthetic lipid membranes has been collected from nuclear
magnetic resonance (9,10), X-ray and neutron diffraction (11,12),
and infrared spectroscopic studies (13) indicating that the P-N
vector lies approximately parallel to the membrane surface.
Since the net dipole of interest is that quantity perpendicular
to the membrane plane, these results imply that the majority of
the dipolar charge is contributed by the two carbonyl moieties.
Further contributions to the net dipole originate from water
molecules which are present due to headgroup hydration (14).
Direct measurements of lipid monolayer dipolar potentials at
air/water (15) and hydrocarbon/water (16) interfaces using
radioactive ionizing and vibrating plate electrodes have
indicated that the dipolar contributions of the carbonyls, P-N
and water vectors are approximately 68%, 16% and 16%,
respectively. The actual net perpendicular dipole magnitude is
much more difficult to obtain due to angular orientations,
opposing dipoles and subsurface phenomena (17), but has been
previously estimated to be of the order of 0.5 to 0.7 Debye per
molecule of PC (15). The effect of aligned and packed lipid
dipoles is to create a positive transmembrane potential which for
a monolayer can be hundreds of millivolts in size (15,18) and is
presumably of greater magnitude for a BLM. A schematic
representation of an intrinsic BLM dipolar potential is shown in

Fig. 8. The application of an external potential determines the direction of ion movement. A comprehensive treatment of the effects of dipolar potential on membrane ion permeability has been produced by Szabo (19), from which the following pertinent relations are noted:

A generalized Nernst-Planck diffusion equation representing ion current density, J, through the membrane can be expressed as

$$-J = zFD(x)c(x)\frac{d}{dx}[\frac{\mu_m^\circ(x)}{RT} + \frac{zF\Psi(x)}{RT} + Lnc(x)] \tag{1}$$

assuming a single ion is present in the membrane, where $c(x)$ is the permion concentration and

z = valence of permion

F = Faraday constant

x = position in membrane

$D(x)$ = diffusion coefficient

R = gas constant

T = absolute temperature

$\mu_m^\circ(x)$ = position dependent free energy

$\psi(x)$ = dipolar potential

This equation can be manipulated to lead to the important relation,

$$J \, \alpha \, \exp[\frac{-zF\Delta\psi_o}{RT}] \tag{2}$$

where $\Delta\psi_o$ = change in potential between the membrane interior and the aqueous phase.

Similar expressions have been proposed and used by Haydon (18) and Lundstrom (20). It is assumed that considerable change in membrane current can be obtained by change of ψ_o through selective interactions at the membrane surface. The magnitude of the current change is determined by the initial value of ψ_o in addition to the total alteration of the potential.

We have successfully investigated electrochemically active receptor-like systems based on enzyme-substrate, antibody-antigen, hormone-receptor and polysaccharide-glycoreceptor interactions. Observations derived from non-selective membrane interactions with organic species known to alter dipolar

potentials and from electrochemical dipolar potential probes, coupled with data from the latter receptor systems have established the existence of the dipolar potential alteration hypothesis as a plausible mechanism of receptor action. The fact that dipolar potential alteration is related to certain receptor activity indicates that this relatively simple yet highly selective mechanism should be pursued for practical receptor implementation.

Ion Conduction Through BLM

Few polarizable groups exist in the interior of a cholesterol/PC BLM, explaining the poor conduction properties which have been documented in the literature. The membrane forming solutions employed in this work were purposefully oxidized to create ion conducting membranes. This resulted in the introduction of polar species into the membrane interior, particularly through oxidation of the alkene residues found in the lipid acyl chains. The distribution of such double bonds in phosphatidyl choline can be combined with an estimate of polar group distribution of sterol to prepare an approximation of the probability density of polarizable groups distributed through the membrane. The probability that a molecule in a membrane will contain a functional group at a specified position has been calculated by assuming:

   a) an estimate of lipid and sterol oxidation
   b) a PC/C mole fraction ratio
   c) a symmetric distribution of alkene groups about the
      center of the acyl chains in a typical divinyl methane
      structure
   d) oxidation of the latter to form acyl chains with polar
      hydroperoxide substitutions

The simple model employed presents evidence for four localized high charge density areas suitable for ion binding or Born energy reduction through dipolar shielding effects. These areas are interspersed with a general "smeared out" increase of the internal dielectric constant due to the presence of polar groups.

Eyring has proposed an absolute rate theory of membrane ion permeation which treats the problem as a migration over a series of activation energy barriers (21). The size of the activation energy barrier to transmembrane ion permeation, the magnitude of reduction of this barrier by selective interactions and the location of the rate determining step to ion transport for the purpose of relocation and placement of a selective interaction must be determined to properly develop and maximize the analytical sensitivity of a BLM-based sensor. The relationship of unmodified BLM residual current to temperature provides thermal data suitable for analysis with the Arrhenius equation to determine the activation energy E_a :

$$Ln(I_r) = \frac{-E_a}{RT} + constant \qquad (3)$$

where I_r = residual current, T = absolute temperature and R = appropriate gas constant. Figure 9 represents the data in standard graphical Arrhenius format, where the slopes of the resulting linear plots are proportional to the total activation energy barriers. The linearity of each plot is expected due to the small temperature range investigated. The barrier can be represented as an electrical potential by substituting the equation:

$$I_r = I_o exp(-eQ/KT) \qquad (4)$$

where Q = potential energy barrier, e = standard charge and K = Boltzmann constant. This results in barriers of 1150 mV and 900 mV (error ± 5%) for mole fraction cholesterol contents of 0.66 and 0.80, respectively. The size of this barrier indicates that ions probably pass through the membrane in a partially hydrated form. The barrier is a function of both the electrostatic (estimated to be about 400-600 mV in magnitude by calculations and experiment) and the chemical potential energies, either of which may be reduced for signal generation. It is proposed that the oxidized sterol products will have the most influence on surface dipole potentials and lipid packing characteristics, while the oxidized alkene residues induce reduction of the hydrocarbon chemical potential energy barrier to ion flux.

Chromatographic and Thermal Investigation of Oxidation

The incorporation of cholesterol into BLM offers alteration of intra-membrane physical chemistry in addition to increased planar membrane strength and stability. The sterol can readily undergo an oxidative transformation to produce entities with increased polarity. At least 50 derivatives are involved in the autoxidation of cholesterol through processes involving light and heat (22), of which approximately 5-10 are produced in sufficient quantities to be deemed important. The complex mixture may contain substances of both membrane stabilizing and destabilizing influence though the former seems predominant. Analysis indicates that conversion of the cholesterol hydroxyl group and B-ring unsaturation to more polar moieties assists membrane stabilization. Formation and rearrangements of carbonyl, peroxide and hydroxyl residues at the A and B ring positions are common, and peroxides and other polar residues can form on the hydrocarbon chain (22). Generally oxidation initiation and propagation processes act on cholesterol to produce reactive intermediates such as hydroperoxides and cholest-5-ene-3-one,

which eventually react to form the volatiles, alcohols, ketones, aldehydes, alkanes, olefins and acids commonly observed.

Thin layer chromatography is the most versatile simple analysis method suitable for general separation of the major oxidation products of both lipid and cholesterol. The results of the separation methodologies attempted indicate that under optimum conditions, up to three lipid and 15 cholesterol oxidation products can be qualitatively isolated and identified.

Quantification can be established on a relative basis of total sterol by techniques such as gas chromatography. The problems of separation encountered for mixtures of closely related sterol compounds are alleviated through implementation of high efficiency capillary columns. The inherent sensitivity of the flame ionization detection system is sufficient to measure nanogram quantities of the various sterols. For analysis, the sterol samples can be directly injected into the chromatograph in a solvent such as chloroform, though the thermal instability of the common cholesterol products at the temperatures required to vapourize the sample is such that often a thermal decomposition occurs (23).

A new method suitable for concurrent quantitative analysis of the extent of lipid and sterol oxidation has been developed during this work. The procedural details have been described in the experimental section, indicating that sample preparatiion does not include preseparation of lipid and sterol. The process provides volatile methyl esters of the lipid acyl chains which can be chromatographed with the oxidized sterols. At constant injection and column temperature, a reproducible chromatogram of lipid methylate, and native and pyrolyzed sterol products evolves as shown in Figure 10. An identification of the eluted species has been attempted through use of gas chromatographic analysis of TLC preseparated sterol products and the use of known standards. The results of this tentative identification are listed in Table 2. The development of a facile, rapid and quantitative technique for evaluation of sample oxidation provides the opportunity for correlation of electrochemistry with oxidation. This has been applied to test the hypothesis relating oxidation to electrostatic and chemical potential energy barrier alterations as determined by thermal electrochemical properties of BLM. The results of the initial studies are illustrated in Figure 11 and clearly indicate the validity of the hypothesis.

Note that oxidation of lipid/cholesterol predominantly occurs in the membrane forming solution rather than in the BLM. Investigation of the autoxidation of egg derived lecithin in the organic solvent chlorobenzene and as BLM vesicles in aqueous 0.1 M NaCl maintained at 30°C and one atmosphere pressure of oxygen confirmed that the reaction rate was greatly reduced in the aqueous phase (24). This was probably a consequence of the high viscosity of the BLM interior, resulting in a ratio of efficiency of chain initiation for oxidation of 0.66/0.043 for the organic/aqueous phases respectively (24). This indicates that

the gas chromatographic method employed here is a suitable
technique for quantitative evaluation of the oxidation products
in experiments using BLM.

SUMMARY OF THE ANALYTICAL POTENTIAL OF BLM SELECTIVE SENSORS

Processes Controlling the Analytical Response

The previous section demonstrated the origins of ion
conductive mechanisms which are required for signal generation in
the absence of membrane intrinsic protein based pathways. The
principle of selective sensing is most simply incorporated into
the BLM by defining the receptor at the membrane surface. The
receptor stimulant interaction should decrease an
electrostatically controlled rate-determining ion transport
barrier which limits transmembrane conduction by impeding ion
access to some form of facilitated transport pathway. To further
simplify the system, such a pathway should be based on intrinsic
properties of the molecules which form the membrane (implying
that control of chemistry is of paramount importance if
reproducibility is to be attained). A more complicated
electrostatically controlled process involving activation or
regulation of protein based conduction systems is also feasible.

The BLM sensor can be considered a useful prototype of a
selective trace organic sensor which has numerous advantages over
conventional techniques such as biocompatibility, selectivity,
low cost, ease of use and portability. The system can be
sensitive and usually has a limit of detection of 10^{-6} M or
better for simple non-selective membrane interactions. Receptor
systems investigated in this work indicate detection limits of at
least 10^{-8} M for the analyte of interest. Presently the lowest
detection limit has been obtained for non-selective interaction
with an ion transport complex known as valinomycin, and is in the
range of 10^{-12} to 10^{-13} M. It is important to note that
experimentally it is possible to integrate some agents such as
valinomycin into BLM and thereby increase detection limits.
Similarly, such a process makes it possible to measure the
electrochemical response of one molecule if for example it has
the capability to produce a single highly conductive
transmembrane pore. The condition of integration places a new
dimension on the concept of limit-of-detection since it becomes a
time dependent factor, where previously it has only been
concentration dependent. The minimum detectable signal for BLM
sensors will always be determined by residual current magnitude
controlled by intrinsic conductance properties and effects due to
contamination. The techniques of polarography and Auger electron
spectroscopy indicate that optimization of signals from BLM
sensors may involve differentiation of the primary ion current
response to eliminate background interference. Maximum
detectable concentrations are determined by saturation of:

receptor sites, kinetic processes, membrane surface density or ion conductive pathways. Further control of sensitivity can be achieved through amplification processes coupling concerted alterations of chemical and electrical potential barrier decreases.

Sources of Selective Receptors

The design of receptors for BLM can be accomplished in numerous ways, the most useful of which involve:

a)   extraction from natural sources as employed in this work
b)   modification of existing enzyme-substrate or antibody-antigen systems
c)   synthesis via biochemical and genetic engineering routes
d)   synthesis from first principles.

Extraction of natural receptors can be a tedious time consuming process prone to failure. The receptor activity after chemical processing is usually decreased and can be easily destroyed. However this process has been the most successful route for obtaining active receptors in the laboratory. Many enzyme-substrate and antibody-antigen type systems exist but cannot be incorporated into BLM due to polarity, size or steric problems. Recent work has demonstrated that either protein component of such systems can be reacted with long chain hydrophobic molecules to form amide bonded acyl chains which assist membrane incorporation through hydrophobic interactions without substantially altering biochemical activity (25). The relatively new area of genetic engineering has made possible the development of a wide range of proteins on a reasonable production scale. Monoclonal antibodies may be the first of such manufactured proteins to be used in BLM.

One important aspect of this work is that some of the basic criteria of receptor structure, membrane position and mechanism of electrochemical action necessary for proper receptor function have been established. Presently more work is required in this area, but the feasibility of synthesizing receptors in the laboratory for specific targets has been demonstrated. This will become an area of substantial importance for gas sensing technology since the opportunity to finally obtain selective receptors for gases of interest has come of age.

Practical Implementation of Receptors

Since BLM are not practical matrices for routine laboratory use of selective sensors, the integration of receptor activity with stabilized ordered lipid films developed by preparative Langmuir-Blodgett deposition techniques is being investigated. It is antticipated that such deposition techniques for formation

of appropriate stabilized films will become well developed over the next few years. The technique will require the use of a vibration free device for dip casting of lipid films onto a suitable substrate, followed by polymerization to stabilize the lipid structure. Knowledge concerning receptor positioning and function will be critical for designing a useful sensing membrane. Preliminary work has demonstrated that organized lipid films can be deposited on hydrogels, the latter being used to replace the present conventional solution compartments required for BLM support and the electrochemical cell. Microelectrodes implanted into the gel assist in device miniaturization and allow the sensing membrane to be employed in an identical operational configuration to that described for BLM in this work. Receptors embedded in stablized membranes which maintain the electrochemical advantages of the present system will allow testing and implementation of the first practical biosensors.

ACKNOWLEDGEMENTS

REFERENCES

1. M. Thompson, U.J. Krull and P.J. Worsfold, Anal. Chim. Acta, 1980: 117, 121.

2. P. Mueller, D.O. Rudin, H. Ti Tien and W.C. Wescott, Nature, 1962: 194, 979.

3. M. Thompson and U.J. Krull, Anal. Chim. Acta, 1983: 147, 1.

4. E.A. Liberman and V.P. Topaly, Biophysics, 1969: 14, 477.

5. J.A.G. Taylor, J. Mingins and B.A. Pethica, J. Chem. Soc. Faraday Trans., 1976: 72, 2694.

6. H. Ti Tien, "Bilayer Lipid Membranes", Marcel Dekker, New York, 1974.

7. N. Pelick and V. Mahadevan in "Analysis of Lipids and Lipoprotein", Am. Oil Chem. Soc., Champaign, Ill., 1975, pp. 29.

8. J.W. Ross, J.H. Riseman and J.A. Krueger, Pure Appl. Chem., 1973: 36, 473.

9.   H. Hauser, W. Guyer, I. Pascher, P. Skrabal and S. Sundell, Biochemistry, 1980: 19, 366.

10. J. Seelig and J.L. Browning, FEBS Lett., 1978: 92, 41.

11. D.L. Worcester and N.P. Franks, J. Mol. Biol., 1976: 100, 359.

12. G. Buldt, H.U. Gally, J. Seelig and G. Zaccai, J. Mol. Biol., 1979:, 134, 673.

13. A. Akutsu, Y. Kyogoku, H. Nakahara and K. Fukuda, Chem. Phys. Lipids, 1975: 15, 222.

14. G.L. Jendresiak and J.C. Mendible, Biochim. Biophys. Acta, 1976: 424, 149.

15. F. Paltauf, H. Hauser and M.C. Philips, Biochim. Biophys. Acta, 1971: 249, 539.

16. K.C. Sehgal, W.F. Pickard and C.M. Jackson, Biochim. Biophys. Acta, 1979: 552, 11.

17. G.L. Gaines, Jr., "Insoluble Monolayers at Liquid-Gas Interfaces", Interscience, New York, 1966, pp. 188.

18. D.A. Haydon, N.Y. Acad. Sci. Ann., 1975: 264, 2.

19. G. Szabo in "Extreme Environment; Mechanism of Microbial Adaptation", Academic Press, New York, 1976.

20. I. Lundstrom and C. Nylander, J. Theor. Biol., 1981: 88, 671.

21. P. Lauger and B. Neumcke in "Membranes", G. Eisenman, ed., Marcel Dekker, New York, 1973, Vol. 2, pp. 32.

22. L.L. Smith, "Cholesterol Autoxidation", Plenum Press, New York, 1981.

23. J.E. Van Lier and L.L. Smith, Anal. Biochem., 1968: 24, 419.

24. L.R.C. Barclay and K.U. Ingold, J. Am. Chem. Soc., 1981: 103, 6478.

25. V.P. Torchilin, V.G. Omel'Yanenko, A.L. Klibanov, A.I. Mikhailov, V.I. Gol'Danskii and V.N. Smirnov, Biochim. Biophys. Acta, 1980: 602, 511.
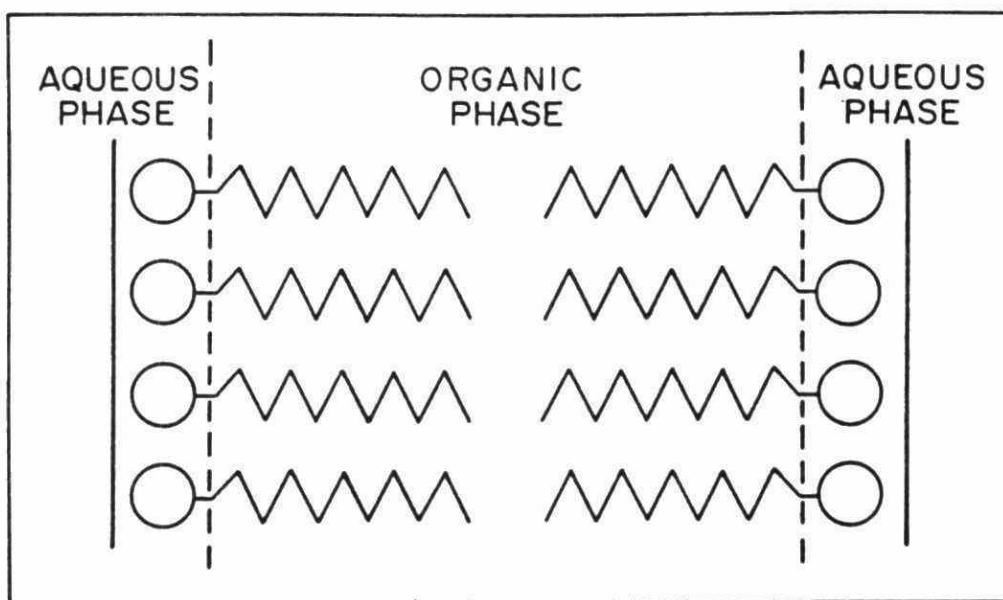
BLM cross-section

Figure]: Schematic representation of a Bilayer Lipid Membrane.
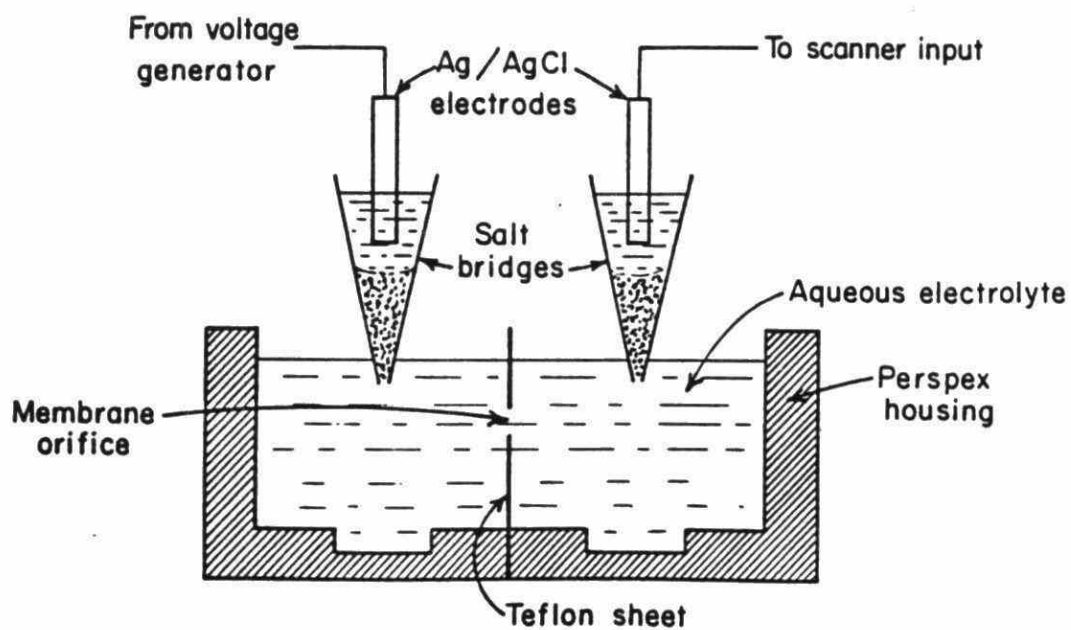
Figure 2: Cell used for electrochemical study of BLM.
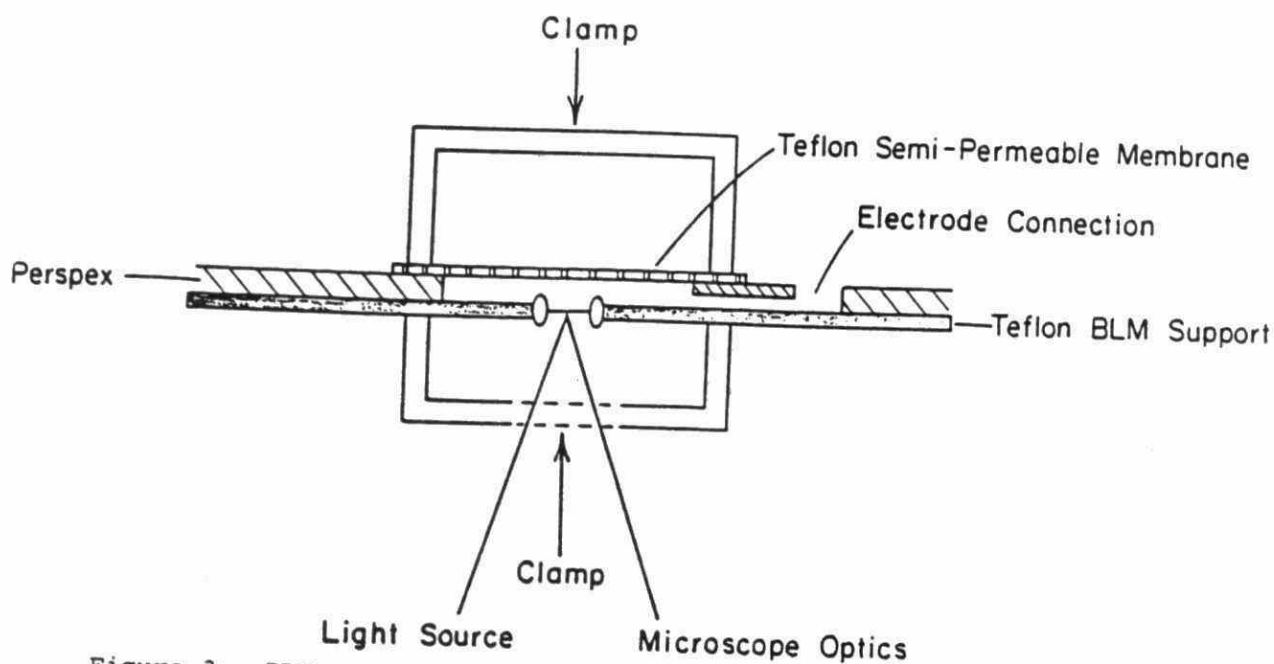
Figure 3: BLM support system used in the ammonia gas detection cell.
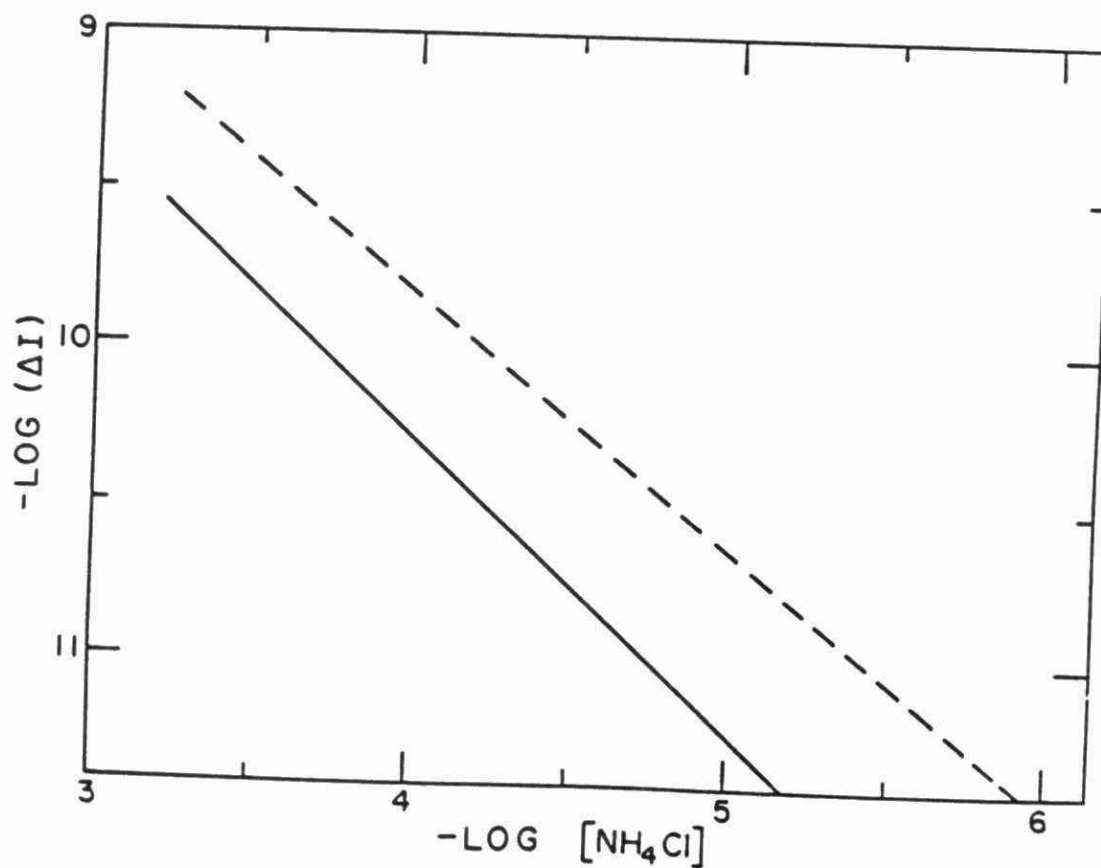
Figure 4: Concentration response curves for a nonactin/LiCl system to NH_4Cl. —— $10^{-3}M LiCl$, ---- $10^{-4}M LiCl$.
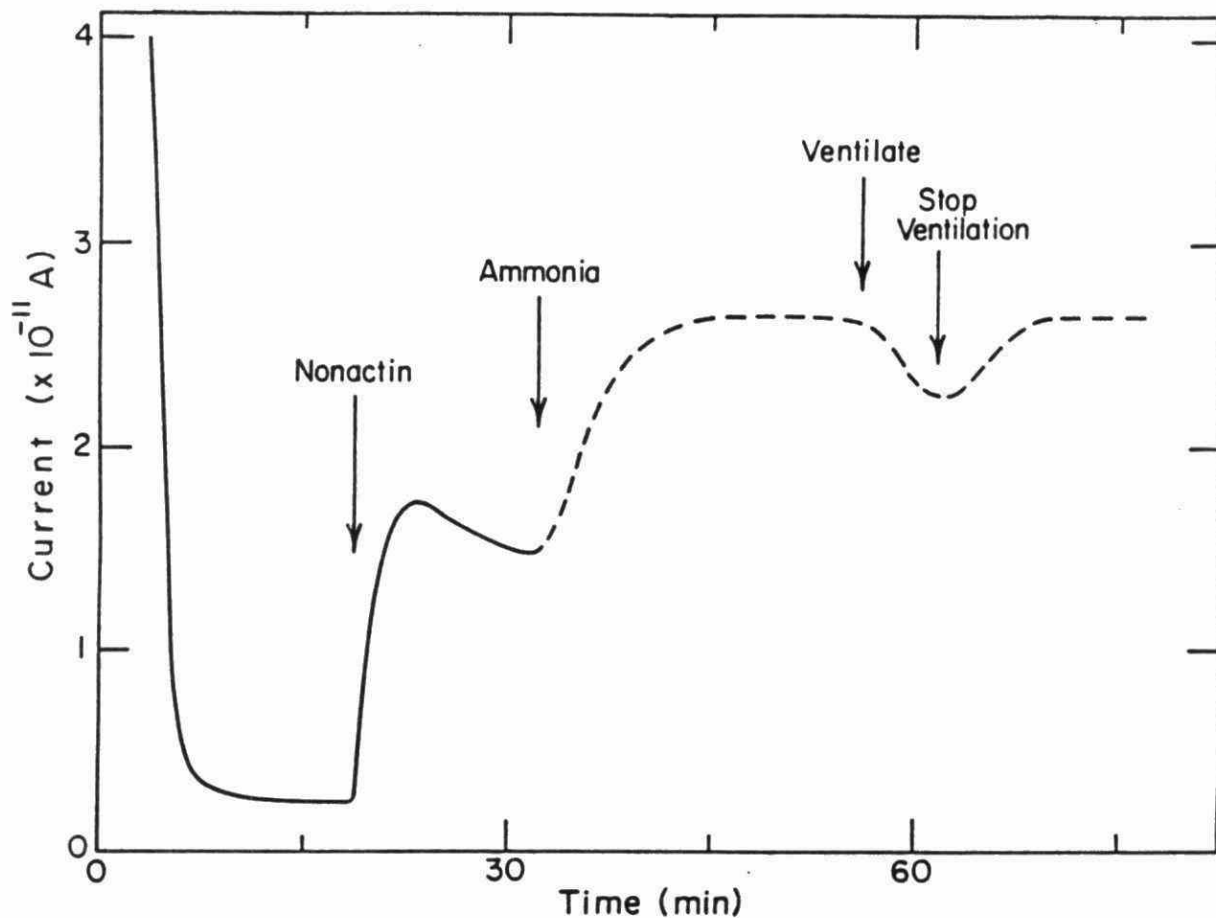
Figure 5: Sensing of concentrated ammonia gas with cell described in Fig. 3. (Nonactin = 10^{-3}M, LiCl = 10^{-4}M, +25 mV)

$$M_{NH_3}/sec = D_{NH_3} \cdot A_{BLM} \cdot \frac{([NH_3]_0 - [NH_3]_i)}{H_{Teflon}}$$

$$M_{NH_3}/sec \longrightarrow M_{NH_4^+}/sec$$
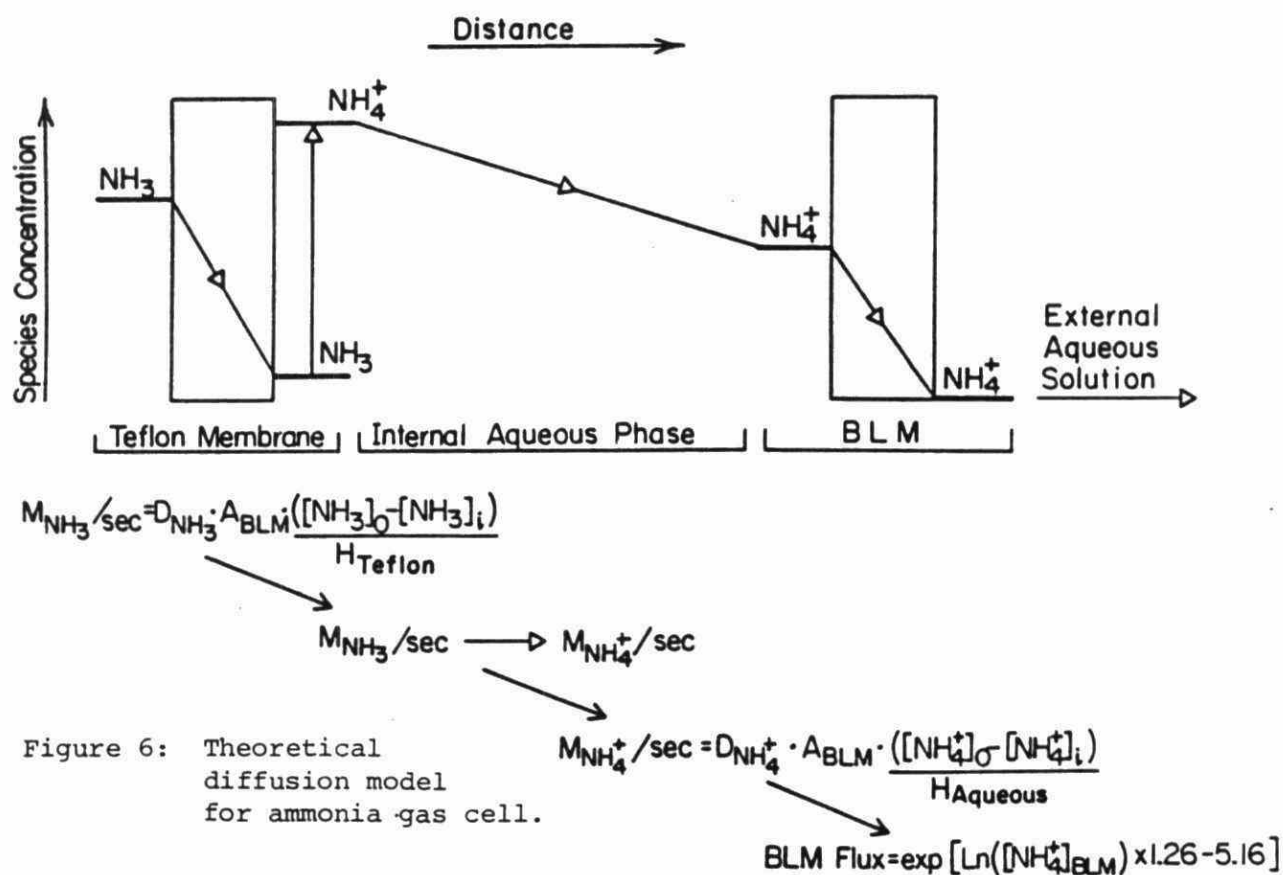
Figure 6: Theoretical diffusion model for ammonia gas cell.

$$M_{NH_4^+}/sec = D_{NH_4^+} \cdot A_{BLM} \cdot \frac{([NH_4^+]_0 - [NH_4^+]_i)}{H_{Aqueous}}$$

$$BLM\ Flux = exp\left[Ln([NH_4^+]_{BLM}) \times 1.26 - 5.16\right]$$

Figure 7: Relationship of transmembrane ammonium ion flux to interfacial ion number density.
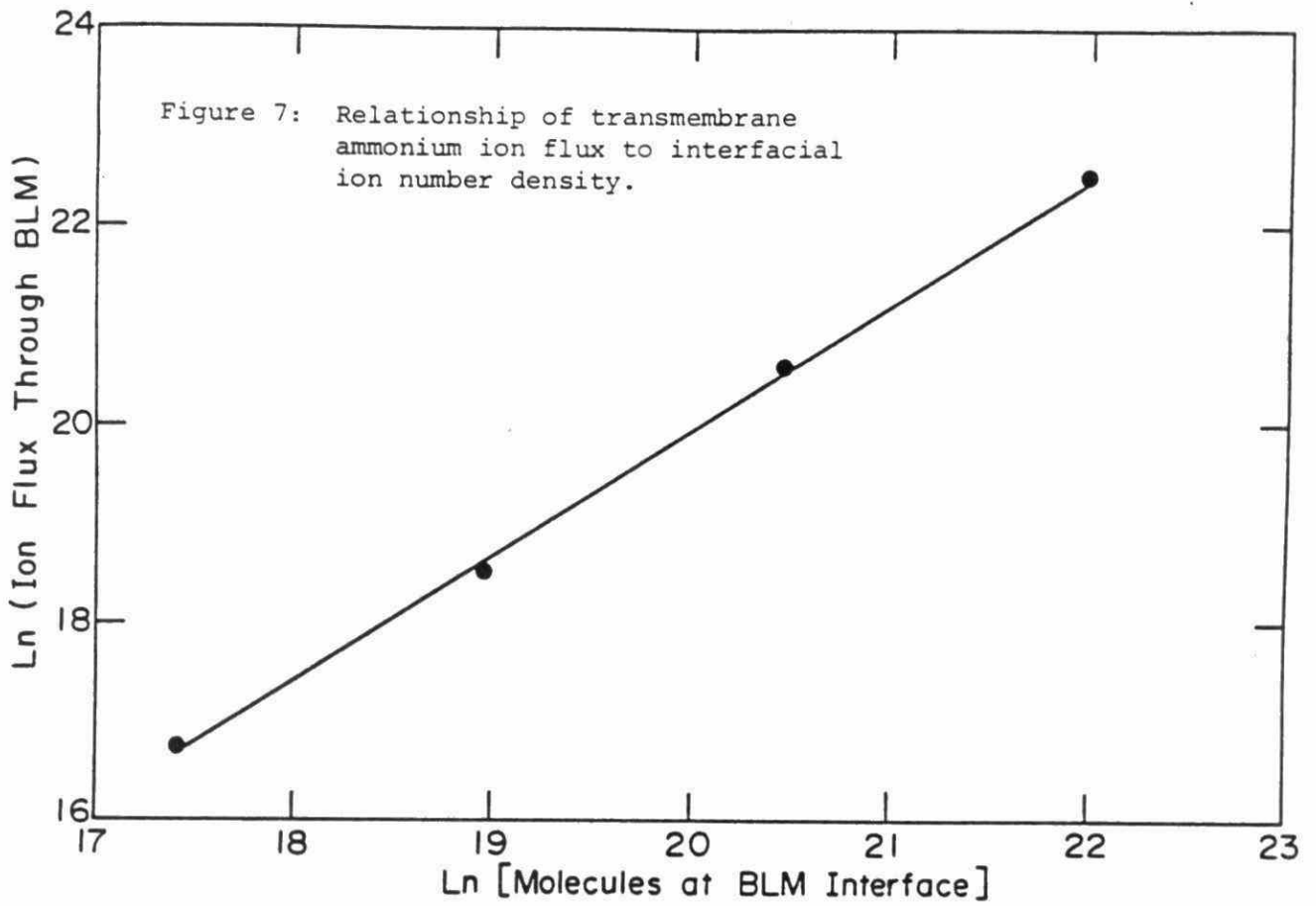
Figure 8: Trapezoidal representation of transmembrane dipolar potential, applied potential and unilateral dipolar potential reduction.

Figure 9: Arrhenius plot of thermal data for BLM transmembrane ion flux. $\bullet - \chi_{cholesterol} \sim 0.66, x-\chi \sim 0.80$.

Figure 10: Capillary gas chromatogram employed for analysis of lipid/sterol oxidation. Conditions and identifications in Table 2.
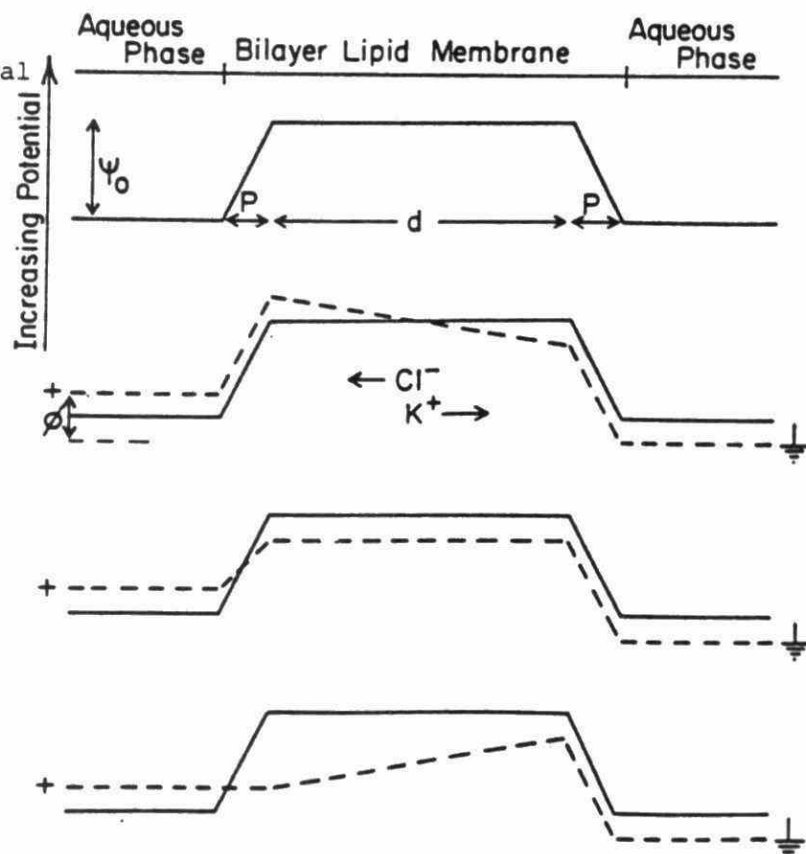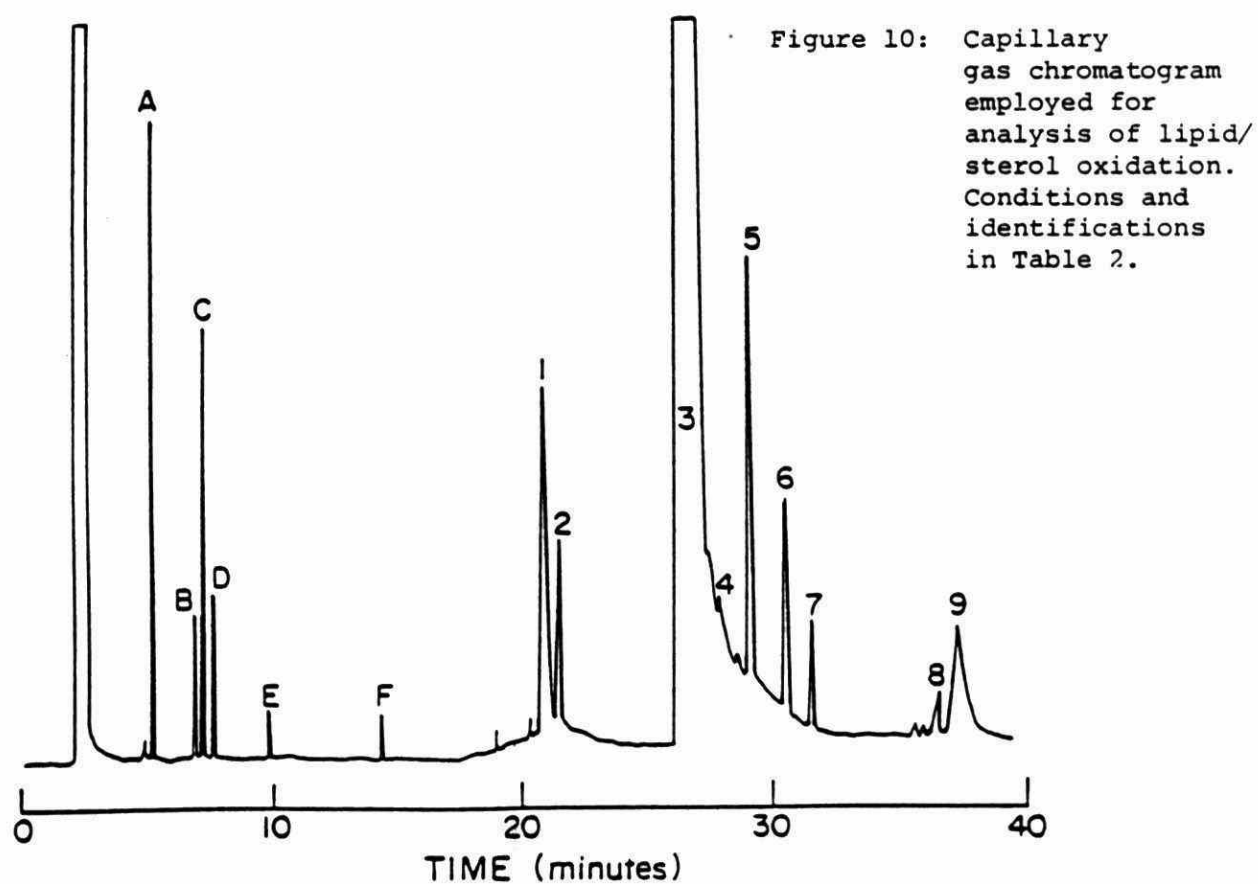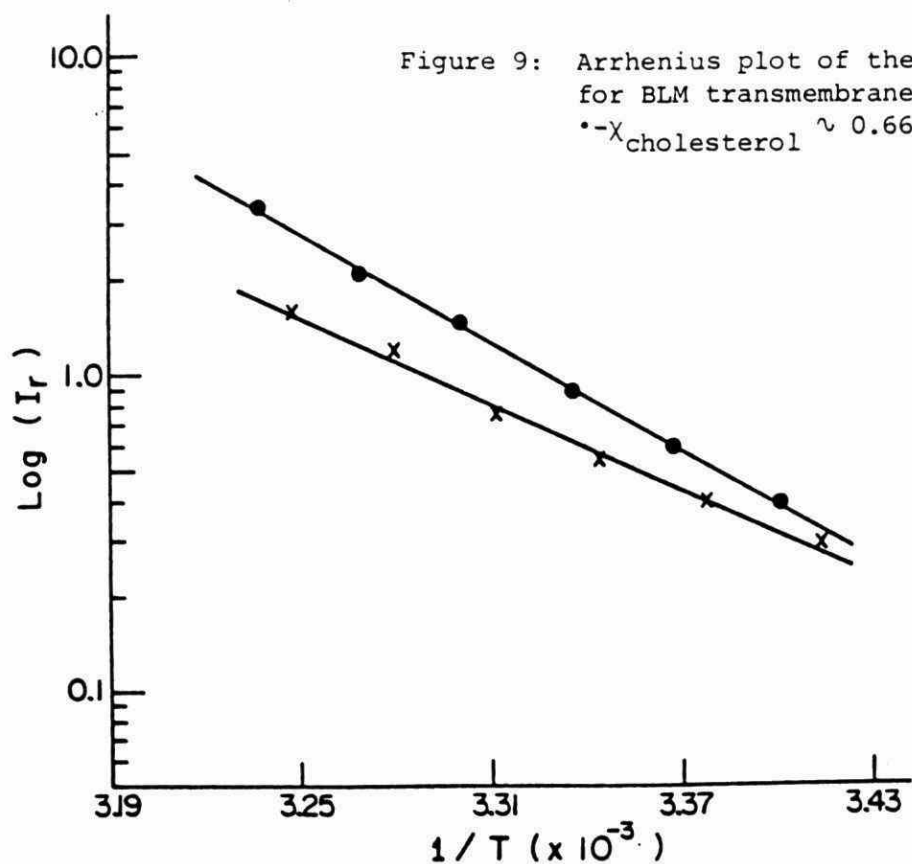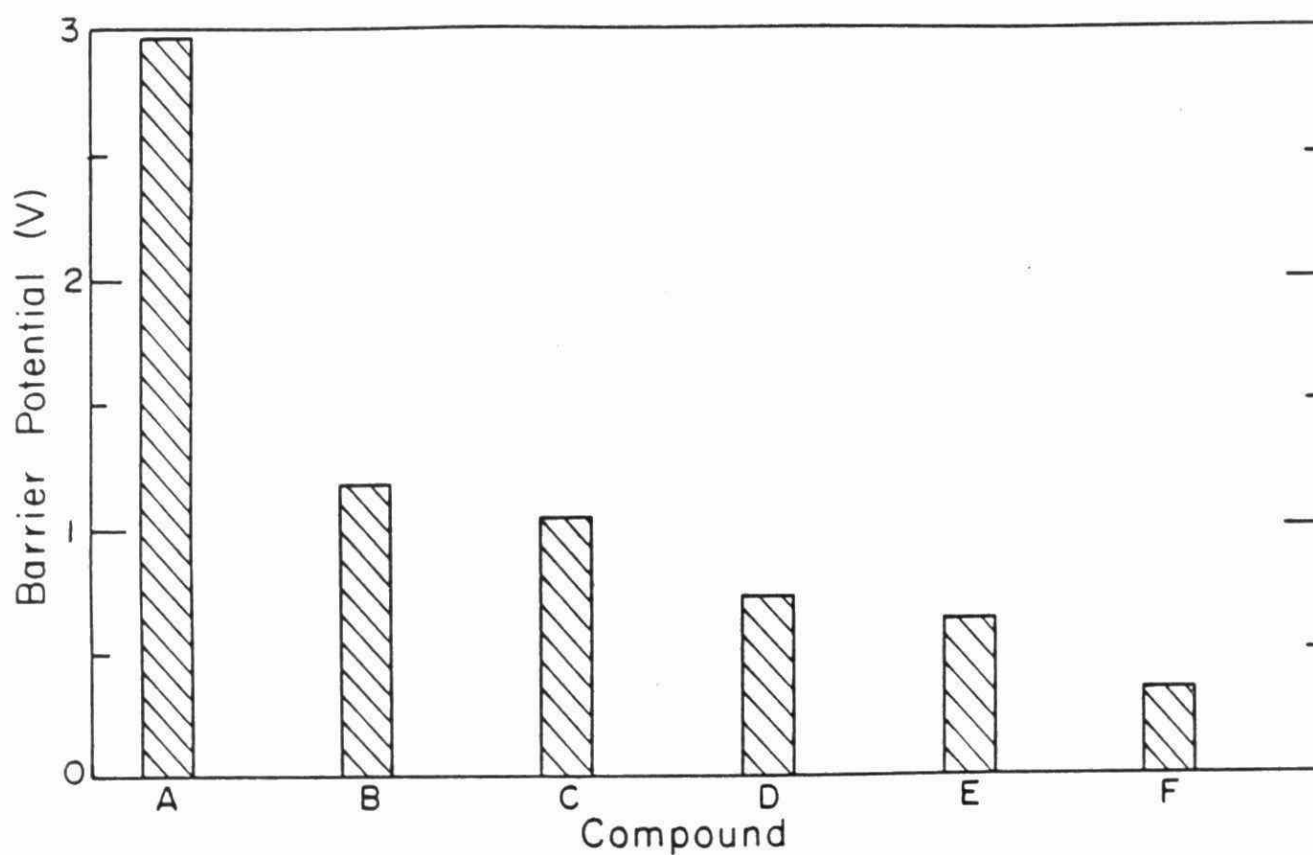
Figure 11: Arrhenius activation energy barrier to transmembrane ion flux as determined by cholesterol oxidation products ($\chi_{sterol} \sim 0.6$)

Compound		Identification
A	-	7α-hydroxycholesterol
B	-	cholesterol
C	-	5,7-cholestadien-3β-ol
D	-	5α-cholestan-3α,5,6β-triol
E	-	7-ketocholesterol
F	-	5,6-epoxycholestan-3β-ol

TABLE 1 : Relative Antibiotic Selectivities for Univalent Cations

| Antibiotic | $\text{Log[Permeability } x^+/\text{Permeability } K^+]$ | | | |
	Li^+	Na^+	K^+	NH_4^+
Gramicidin	-0.9	-0.2	0	+0.1
Valinomycin	-6	-5	0	-2
Nonactin	-3	-2	0	+0.75
Crown Ether *	-2	-1.5	0	-0.5

*bis(t-butyl)dicyclehexyl-18-crown-6

Table 2: *Identification of products shown in capillary gas chromatogram of Fig. 10.

Lipid Acyl Methylate Product	Alkene Identification	Sterol Product	Tentative Identification
A	— 16:0	1	— 7α-hydroxycholesterol
B	— 18:2	2	— 5,6-epoxycholestan-3β-ol
C	— 18:1	3	— cholesterol
D	— 18:0	4	— cholesta-3,5-diene
E	— 20:4	5	— cholesta-3,5-dien-7-one
F	— 22:6	6	— 5α-cholestane-3α,5,6β-triol
		7	— 5,6-epoxycholestan-3β-ol
		8	— 5α-cholestane-3α,5,6β-triol
		9	— 7-ketocholesterol

*Operating Conditions: Temperature programmed from 200 to 280°C @ 4°/min. from 0.5μℓ injection. Injector temperature ∿ 325°C. Helium carrier gas flow ∿ 0.5 ml/min.

METHODS FOR SAMPLING AND ANALYSIS OF

ASBESTOS AIR POLLUTION IN ONTARIO

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Abstract

Asbestos, a known fibrogenic and carcinogenic material, has been identified in the past as the problem associated with occupational environments. It is estimated that almost half of all occupationally related cancers are caused by asbestos. Asbestos disease of clinical significance is probably undetectable at low levels of exposure. Some malignancies, particularly mesothelioma appear to be associated with surprisingly low levels of exposure. This accounts for the importance of asbestos pollution of outdoor air as well as of indoor air (eg. schools and offices) of Ontario.

This presentation will report on the ongoing research funded by the Ministry of the Environment's Air Resources Branch. The rationale for research issues will be discussed. Data on the examination of methods for sampling and analysis of low levels of asbestos including various electron microscopic techniques will be presented.

INTRODUCTION

Asbestos, a known fibrogen and carcinogen, has been identified in the past as a problem associated with occupational environments (factories, mines, construction, etc.) (1-7). It is estimated that almost half of all occupationally related cancers are caused by asbestos (8).

Diseases associated with asbestos are generally dose dependent. Some malignancies, particularly mesothelioma, appear to be related to surprisingly low levels of exposure accounting for the importance of asbestos pollution in outside air.

Because of its carcinogenic potential, asbestos contamination of outdoor air as well as indoor air (eg. schools and buildings) has now taken on a much greater importance. It is necessary that methods be developed and data collected to accurately detect and quantify asbestos in these situations so that the extent of the problem can be more precisely assessed. It is a well known fact that the present environmental guidelines for asbestos in Ontario and elsewhere (See Table 1) have been arrived at by extrapolation of occupational exposure data (9,10) and by comparison of outdoor background levels with those found in asbestos insulated buildings (11). The environmental guidelines are based on evaluations using electron microscopy.

In the occupational setting, health effects were related to the fibre levels obtained by traditional occupational exposure measurement techniques (the membrane filter method using optical microscopy, thermal precipitator counts, impinger counts, etc.).

The fibre counts obtained by phase contrast optical microscopy (PCOM) differ greatly from those obtained by electron microscopy. (EM). It has been suggested that counts obtained by electron microscopy could be 50 to 100 times higher than those counted using the optical method on the same air sample (12,13). In a recent study, a similarly high ratio of EM to optical fibre counts in air samples has again been demonstrated (14).

The issue of fibre dimension, that is, sizes visible by EM and those visible by PCOM, is shown graphically in Figure 1. This is extremely important if the carcinogencity of asbestos fibres is related to fibre morphology. The fibres that are thin (less than 1.5 µm) and longer than 8 µm are suspected of being the most carcinogenic (15). A vast majority of such thin and long fibres could not be detected by optical microscopy but can only be seen by Electron Microscopy (See Figure 1). The proportion of fibres in the different size ranges depends on the type and operation involved. Furthermore, the PCOM method may include other non-asbestos fibrous material. Accurate identification and quantification of asbestos in outdoor air can only be reliably obtained using EM methods.

At present, data does not exist that relates low asbestos fibre levels obtained by EM to health effects. However, a grading of hazard potential can be developed if the following data is accumulated:

    i)   an inventory of fibre levels in outside air by EM,

    ii) in selected occupational exposure situations, air
             samples analyzed by both EM and PCOM.

A correlation between EM and PCOM fibre counts could be established for specific occupational settings and fibre types. The health effects based primarily on optical counts could then be related to EM fibre counts. This is one feasible way of obtaining estimates of the risk associated with outside asbestos exposure (see Figure 2).

It is clear that in order to assess health risks associated with asbestos air pollution, the most important research tasks are:

i) establishment of relationships between fibre counts measured by the Phase Contrast Optical Microscope (traditional occupational method) to those assessed using the Electron Microscope for various operations and processes involving different types of asbestos.

ii) establishment of precise and accurate EM methods to evaluate asbestos air pollution. This should include a proper strategy for air sampling, sample preparation, counting, sizing and identification.

Sample Preparation

Initial research work was concentrated on setting up Electron Microscopic evaluation techniques. This involved looking at both direct and indirect sample preparation methods (Figure 3). Since we are interested in the size distribution of airborne asbestos fibres, we have concentrated on the direct transfer methods. In particular, we chose a modified Ortiz and Isom technique (29) as shown in Figure 3(B). We are using Millipore (mixed cellulose ester) rather than Nuclepore (polycarbonate) filters because of their ease of dissolution and better collection efficiency.

Fibre Counting Strategy

The next step was the establishment of our tentative strategy for counting fibres on the EM. Eight different EM counting strategies (16-23) from 3 countries (U.K., U.S.A., and Canada) were evaluated. All of the strategies were rejected on validity grounds. Most were based on non-random (ie. systematic) selection of counting fields. We have proposed an interim counting strategy that is based on truly random selection of grid openings on a 200 mesh EM Finders Grid (24). The term "at random" requires that every element in a finite population has the same chance of being included in the sample. By employing a table of random numbers, the observer is prevented from introducing his/her own personal preference.

A counting exercise was conducted to examine these and other systematic strategies for grid opening selection, as well as our own

proposed random method. All available openings (maximum of 234 opening on each grid) on 2 low density (0.5 fibres per opening) and 2 low-medium density (2.5 fibres per opening) grids were counted. The mean counts estimated using the systematic strategies were compared to the true mean count based on the 234 openings. The results are shown in Table 2. It appears from our data that most of the "non-random" strategies were relatively poor predictors of the true concentration. The cross method (20) gave good estimates but at the expense of counting almost 50 openings. Our random approach was assessed by simulating the selection of 5, 10, 15, 20, 25 and 30 grid openings at random from the 4 grids. Accuracy was excellent for all sample sizes while precision was function of the number of openings and came very close to the expected standard errors based on Normal sampling theory. The computer simulation was also useful in determining the optimal sample size. This is discussed in the next section.

Sampling Strategy

In an environmental sampling program, it is important to consider the variation that may be found within a given sample and between samples. In order to obtain a reasonable estimate of the true concentration, a strategy has to be formulated to address issues such as how many filters should be exposed, how many grids out of each filter should be taken and how many grid openings or number of fibres must be counted. These issues were examined for low fibre density

(less than 5 fibres per grid openings) environmental samples (24,25).
High inter-filter (between filters) and inter-grid (within filter)
variability was found, suggesting that greater accuracy would be
achieved by exposing more filters and extracting more grids out of
each filter.

The results from the counting trial of all available openings
from several low density grids indicated that the count distribution
followed the Poisson law (based on Chi Square Goodness of Fit Tests),
(Table 3) while the area distribution exhibited clustering effects.
The results of counting an entire grid are shown in Figure 4. Each dot
represents a fibre and the blackened squares represent damaged
openings. Because of this clustering (consistent on all 4 grids),
systematic strategies could be very misleading. The computer
simulation of random sample selection was also used to determine the
optimum number of fields and fibres required for adequate precision.
For the low density samples, counting at least 20 grid openings
(approximately 50 to 100 fibres total) appeared to be quite adequate.

Identification of Asbestos

In outdoor air, asbestos fibres constitute only a fraction of 1% of all airborne materials. Other mineral and man-made fibres may also be present. To evaluate the associated risk of asbestos in outdoor air, positive identification of asbestos fibres is crucial. We have investigated the following identification methods: Morphology on the Transmission Electron Microscope (TEM), Selected Area Diffraction Patterns (SAED) and microchemical (EDAX) analysis of the fibres.

Elemental analysis by EDAX on various types of standard asbestos (Chrysotile, Amosite, and Crocidolite) has been conducted. We have investigated the positional effect for microchemical analysis of fibres by EDAX. Twenty-four fibres from each type (Chrysotile, Amosite and Crocidolite) randomly selected from UICC Standards were elementally analyzed and sized at 5 locations along the fibre: at each end, at the centre, and at a position equidistant from the centre to each end. A summary by fibre type and elemental ratios (relative to Silicon) is displayed in Table 4. The elemental ratios were analyzed using a repeated measures analysis of covariance - type and position as factors, and overall fibre length and positional diameter as covariates. For all types, fibre location was not significant. Based on the variance estimates, the centre position was found to be the most stable. The length of the fibre also appeared to have no effect.

Since the Silicon levels were highly correlated with diameter and all elemental values were divided by the amount of Silicon, it was felt that the elemental ratios would not be related to the fibre diameter. This was not the case for the Fe/Si ratio in the Crocidolite fibres. A best fit linear regression relating Fe/Si % to the reciprocal diameter had the form:

$$Fe/Si \ \% \ = \ 82 - 0.89 \ (1/Diameter)$$

with R^2 = 42% and MSE = 7.2 .

Relationship Between PCOM and EM counts

Experimental work is currently underway examining occupational and environmental samples using both PCOM and EM methods. Our tentative strategy involves dividing an exposed 37 mm diameter filter into 8 equal pie-shaped wedges: 2 to be used for PCOM analysis, 4 wedges for direct EM methods, and 2 for use with indirect EM techniques - all will be randomly assigned. Furthermore, we intend to examine at least 1 grid per sample under the optical microscope. To speed up the counting/sizing process, we are employing a microfiche reader at 32X magnification to examine plate film negatives. The entire grid opening is photographed on the EM at 720X magnification. This approach cuts down on the amount of EM time required and reduces observer fatigue while maintaining excellent resolution. We have also developed software on an Apple II+ microcomputer interfaced with a sonic digitizer to measure fibre dimensions directly off the microfiche screen or micrographs.

Biological Relationships

The effects of asbestos air pollution, where the lung is the target organ, can also be evaluated by actual analysis of fibres that are found in the lungs of urban and rural dwellers. Such work involving asbestos in lungs has been done elsewhere (26-28) and should be carried out in Ontario.

CONCLUSION

Since the study's inception, we have established a sample preparation method for EM samples and reviewed various statistical strategies for selecting representative samples. Initial results from our counting experiment suggest that for low density samples, the count distribution is Poisson while the actual area distribution displays clustering. It is imperative that grid openings should be selected for counting at random as suggested in our interim method. Systematic methods of selection appear to underestimate the true mean count.

The work has been successfully initiated in the identification of fibres by microchemical analysis (eg. EDAX) and by selected area diffraction patterns (SAED). Work is in progress in the area of deriving relationships between optical and electron microscope counts so that the extent of health effects of low level asbestos found in outside air could be estimated.

ACKNOWLEDGEMENTS

REFERENCES

1. NIOSH, (1972) Criteria for a Recommended Standard ...
    Occupational Exposure to Asbestos, U.S. DHEW
    Publications, Cincinnati, Ohio

2. NIOSH, (1976) Revised Recommended Asbestos Standard, U.S.
    DHEW Publications, Cincinnati, Ohio

3. WALTON, W.H., (1982) The Nature, Hazards and Assessment Of
    Occupational Exposure to Airborne Asbestos Dust: A
    Review, Ann.Occ.Hyg., 25, 117-247

4. LANE, R.E., (Chairman)(1968) Hygiene Standard for Chrysotile
    Asbestos Dust, Ann.Occ.Hyg., Vol 11, pp 47-69

5. The Final Report of the United Kingdom Advisory Committee on
    Asbestos, 1979

6. SUGAR, S., (1979) Effect of Asbestos in the Canadian
    Environment, A Report from the Associated Committee on
    Scientific Criteria for Environmental quality on the
    National Research Council of Canada, Publ # NRCC 16456

7. ONTARIO MINISTRY OF THE ENVIRONMENT, (1975) Asbestos as a
    Hazardous Contaminant, Report No. ARB TDA 01-75

8. DOLL, R. and PETO,R., (1981) The Causes of Cancer (Oxford
    Medical Publications) Oxford University Press

9. BRUCKMAN, L. and RUBINO, R.A., (1975) Asbestos: Rationale
    Behind a Proposed Air Quality Standard, J.A.P.C.A.,
    Vol 25, No. 12, pp. 1207-1214

10. ONTARIO MINISTRY OF THE ENVIRONMENT (1981), Personal
    Communication with Dr. P. Roberts

11. SEBASTIEN, P., BILLION-GALLAND, M.A., DUFOUR,G., and
    BIGNON, J. (1977) Measurement of Asbestos Air
    Pollution Inside Building Sprayed with Asbestos, English
    Tranitation of French Publication, EPA 560/13-80-026,
    August 1980

12. CHATFIELD, E.J., (1979) Measurement of Asbestos Fibres in
    the Workplace and in the General Environment,
    Mineralogical Association of Canada, Short Course
    Handbook, edited by Ledous, R.L., Quebec, pp 111-157

13. WINER, A.A. and COSSETTE, M., (1979) The Effects of Aspect Ratio on Fibre Counts: A Preliminary Study, Annal.of N.Y.A.S., Vol. 330, pp. 661-672

14. HWANG, C.Y. and WANG, Z.M., (1983) Comparison of Methods for Assessing Asbestos Fiber Concentration, Arch.Env.Health Vol. 38, No. 1, pp. 5-10

15. STANTON, M.F. and LAYARD, M., (1978) The carcinogenicity of Fibrous Minerals, In: Gravett, G.C., LaFleur, P.D., and Heinrich, K.F.J., (eds.) Workshop on Asbestos: Definition and Measurement Methods (National Bureau of Standard Special Publication No. 506)

16. BURDETT, G., LeGUEN, J.P., ROAD, A.P., and ROOKER, S.J., (1980) Comprehensive Methods for Rapid Quantitative Analysis of Airborne Particulates by Optical Microscopy, SEM and TEM with Special Reference to Asbestos Atmospheric Pollution, 1980 Proceedings of the 14th International Colloquium, Paris, France, M.M. Bernarie (Ed.) Studies in Environmental Sciences, Vol. 8, pp. 323-338

17. RICKARDS, A.L., (1973), Estimation of Submicrogram Quantities of Chrysotile Asbestos by Electron Microscopy, Vol. 45, No. 4, pp. 809-811,

18. POOLEY, F.D. and CLARK, N.J., (1979) Quantitative Assessment
     of Inorganic Fibrous Particules in Dust Samples with an
     Analytical Transmission Electron Microscope,
     Ann.Occ.Hyg., Vol. 22, pp 253-271

19. NICHOLSON, W.J. and PUNDSACK, F.L., (1973) Asbestos in the
     Environment, Biological Effect of Asbestos, Gobovski,
     Gilson, Timbrell, and Wagner,(ed.) pp 126-130

20. McMILLIAN, L.M., STOUT, R.G., and WILLEY, B.F.,(1977)
     Asbestos in Raw and Treated Water: An Electron
     Microscopy Study, Environmental Science and Technology
     11(4), pp 390-394

21. SAMUDRA, A.V., HARWOOD, C.F., and STOCKHAM,J.D., (1977)
     Electron Microscope Measurement Of Airborne
     Asbestos Concentrations, A Provisional Methodology
     Manual, EPA. 600/2-77-178

22. ONTARIO MINISTRY OF THE ENVIRONMENT, (1976) An Interim Method
     for the Determination of Asbestiform Mineral Fibre
     Levels in air and Water, pp 1-67

23. ONTARIO MINISTRY OF THE ENVIRONMENT, (1976) An Interim
     Method for the Determination of Asbestos Fibre
     Concentrations in Water by Transmission Electron
     Microscopy, pp. 1-36

24. VERMA, D.K., (1982) Asbestos Analysis Methods and Air

    Pollution in the Outdoor Air in Ontario, Presented

    at Ontario Ministry of Environment Air Resources Branch

    Research Grant Program Annual Seminar, Toronto, Ontario

    (Jan 19/20, 1982)

25. VERMA, D.K., (1983) Methods for Sampling and Analysis of

    Asbestos Air Pollution in Ontario, Presented at Ontario

    Ministry of the Environment Air Resources Branch

    Research Grant Program Annual Seminar, Toronto, Ontario

    (Jan 18/19, 1983)

26. POOLEY, F.D., OLDHAM, P.D., CHANG-HYUN UM, WAGNER, J.C.,

    (1970) The Detection of Asbestos in Tissue, Proceddings

    of the International Conference On Pneumoconiosis, 1969,

    Capetown, Oxford University Press, pp. 108-116

27. LANGER, A.M., SELIKOFF, I.J.,(1971) Chrysotile Asbestos in

    the Lungs of Persons in New York City, Arch.Env.Health.

    Vol 22, pp 348-361

28. CHURG, A., and WARNOCK, M.L., (1980) Asbestos Fibres in the

    General Population, Am.Rev.Resp.Dis., Vol 122, pp 669-

    678

29. ORTIZ, L.W., and ISOM, B.L., (1974) Transfer Technique for

    Electron Microscopy of Membrane Filter Samples, Am.Ind.

    Hyg.Assoc.J., Vol 35, pp. 423-425

TABLE 1

PROPOSED ENVIRONMENTAL GUIDELINES

1) State of New Mexico (1973)
   - 10 ng/m^3 ambient air regulation

2) Bruckman and Rubino (1975), Connecticut, U.S.
   - 30 ng/m^3 using EM examination
   - based on: a) extrapolation of occupational dose-response
               b) relation 2 f/cm^3(OM) $\equiv$ 10^5 ng/m^3(EM)
   - equivalent to 0.0006 fpcc

3) Sebastien et al., (1977), France
   -. 7 ng/m^3 using EM examination
   - based on comparison of outdoor background levels with 21 insulated buildings

4) Ontario MOE, (1981)
   - 0.04 fpcc, fibers > 5μ long by EM count
   - based on 2% of 2 fpcc occupational standard

TABLE 2

MEAN FIBRE COUNTS BY STRATEGY

(NUMBER OF GRID OPENINGS)

GRID NUMBER	TOTAL	QUAD 1	QUAD 2	QUAD 3	QUAD 4	PERIM.	DIAG.	CROSS
A- 03	2.17 (203)	2.15 (53)	2.00 (49)	2.00 (44)	2.47 (57)	1.66 (38)	1.89 (19)	2.45 (47)
A- 02	2.60 (167)	2.75 (44)	2.70 (30)	2.88 (43)	2.16 (50)	·2.04 (29)	2.12 (16)	2.85 (46)
L- 01	0.53 (214)	0.71 (58)	0.40 (50)	0.54 (52)	0.46 (54)	0.39 (28)	0.35 (20)	0.52 (48)·
L- 03	0.32 (170)	0.30 (54)	0.26 (35)	0.14 (21)	0.38 (60)	0.31 (48)	0.07 (14)	0.35 (48)

TABLE 3

Grid Counting Statistical Summary

GRID NUMBER	MEAN	VAR.		Number of Fibers							total	$X^2(df)$
			0	1	2	3	4	5	6+			
A-03	2.172	2.124	Obs 28	42	50	48	26	5	4		203	7.6(6)
			Exp 23.1	50.2	54.6	39.5	21.5	9.3	4.8			
A-02	2.599	2.615	Obs 12	37	34	40	22	14	8		167	2.9(6)
			Exp 12.4	32.3	41.9	36.3	23.6	12.3	8.2			
L-01	0.533	0.595	Obs 128	65	16	3	2				214	0.7(3)
			Exp 125.6	66.9	17.8	3.7						
L-03	0.324	0.457	Obs 129	32	5	3	1				170	2.3(2)
			Exp 123.0	39.8	7.2							

TABLE 4 – <u>ELEMENTAL ANALYSIS OF ASBESTOS</u>: Means (Std.Dev.)

Type	Width(μm)	Length(μm)	Si(cps)	Fe/Si %	Mg/Si %	Mn/Si %	Na/Si %
CHRYSOTILE	.05 (.01)	2.9 (1.2)	8.7 (3.1)	7.1 (2.9)	69.8 (5.6)	---	---
AMOSITE	.55 (.36)	13.8 (8.2)	549.8 (378.8)	92.4 (4.7)	10.0 (6.0)	4.4 (1.1)	---
CROCIDOLITE	.23 (.12)	4.3 (4.3)	86.3 (64.1)	77.1 (5.1)	3.2 (0.7)	---	7.4 (0.8)

FIGURE 1

ASBESTOS FIBER DIMENSIONS
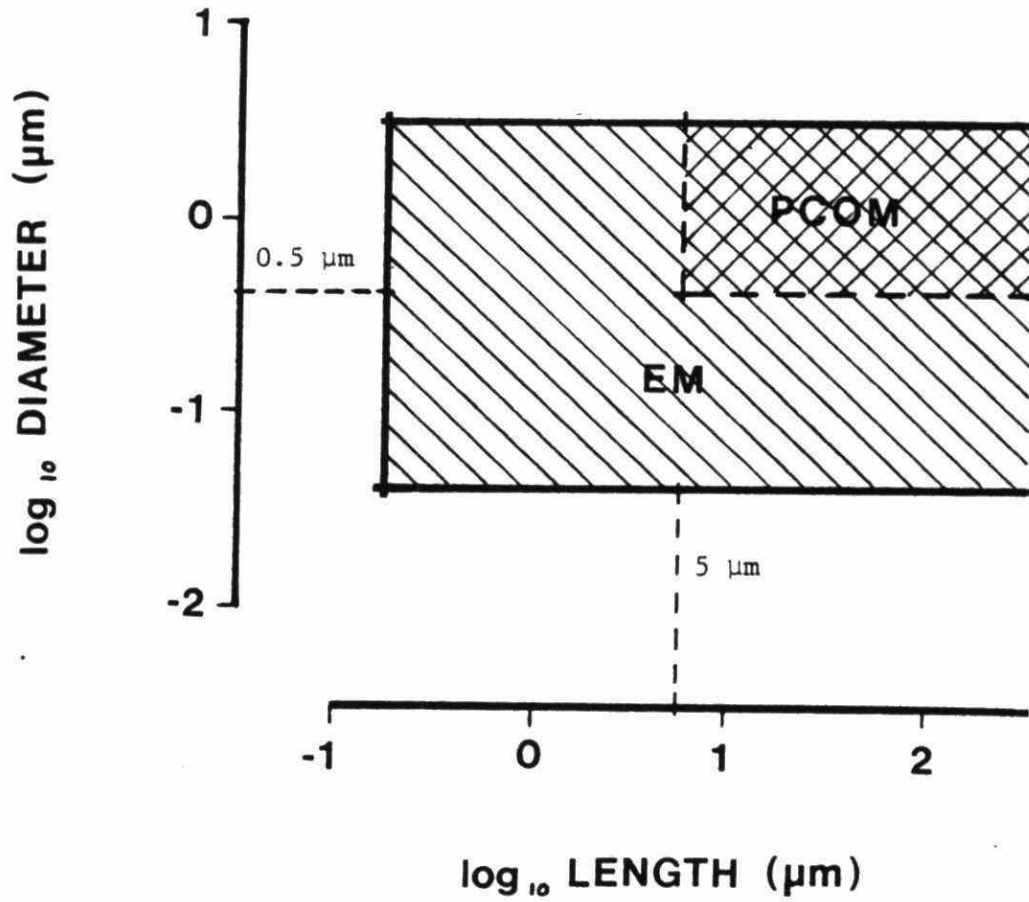
FIGURE 2

ASBESTOS - linear transformation fits well

     - however few data points

     - implies NO THRESHOLD LEVEL

Concentration based on Optical Microscope Counts (OM)

Establish relationship:

$$C_{OM} = f(C_{EM}, \text{ type, operation, ?})$$

For fixed duration:

I

OPTICAL COUNTS
not
reliably
measured
in
this range

Based on
Occupational studies

0.1 fpcc
(Practical Detection Limit)

C_{OM}
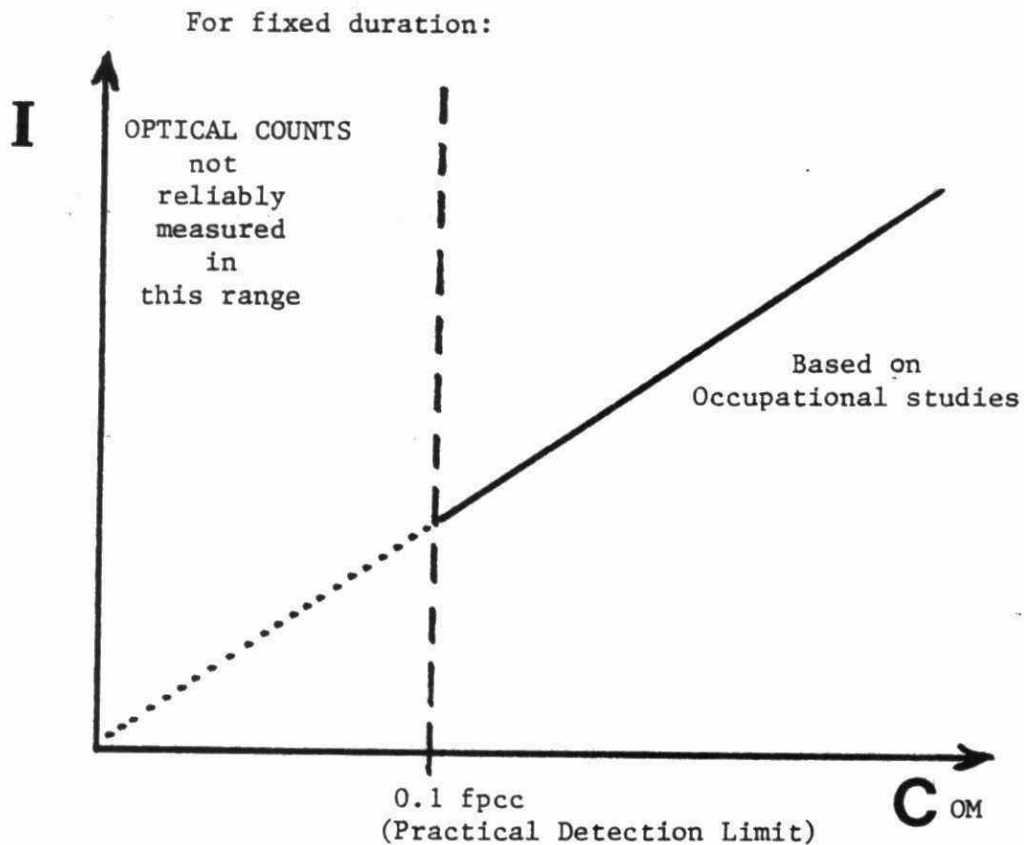
- use C_{OM} - C_{EM} relationship to estimate risk at low levels
- Refinements: 1) allow dose to deteriorate with time

                   (exponential decay)

            2) allow time lag between dose and observed effect.

FIGURE 3

METHODS FOR SAMPLE PREPARATION
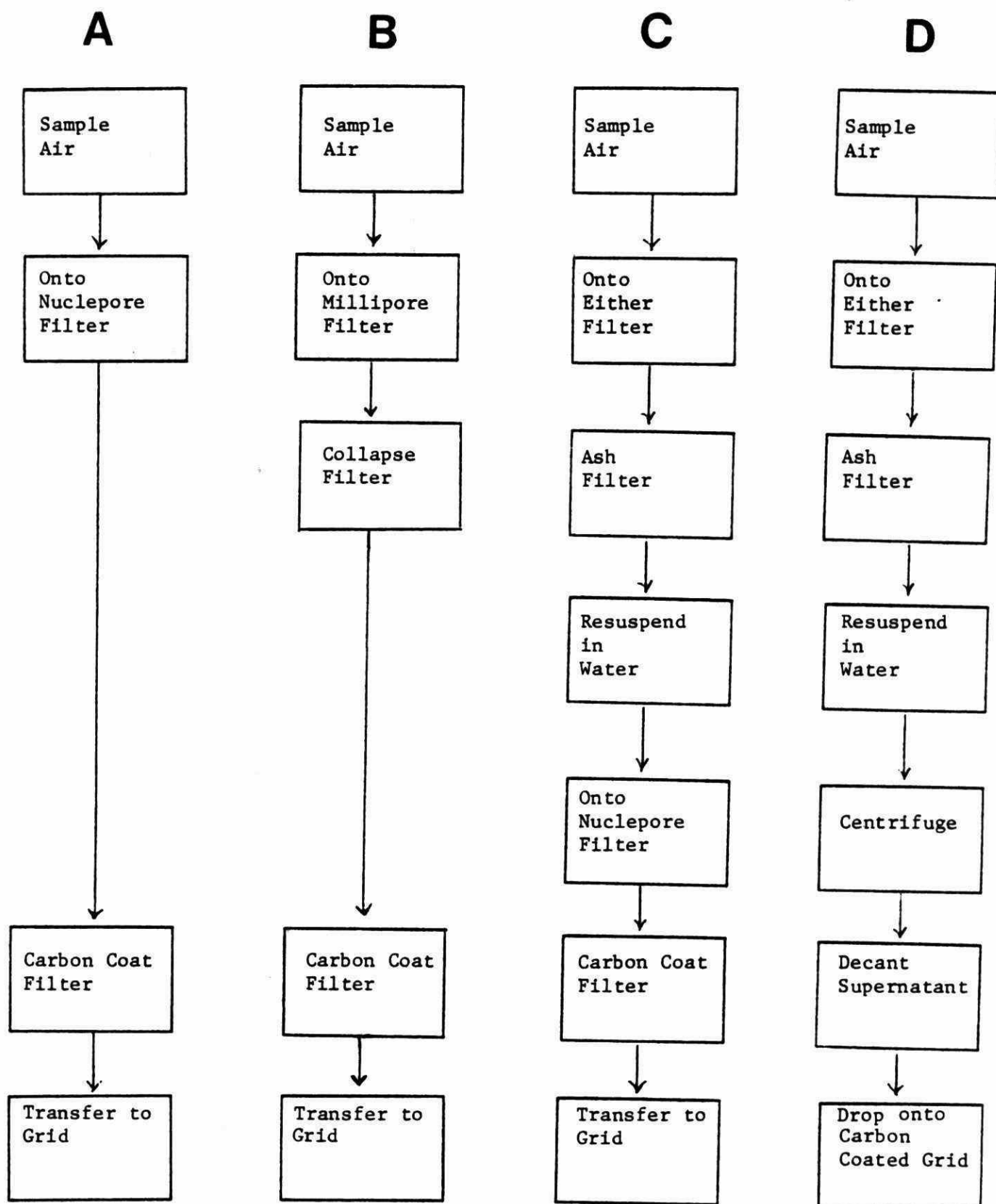
A

Sample Air
↓
Onto Nuclepore Filter
↓
Carbon Coat Filter
↓
Transfer to Grid

B

Sample Air
↓
Onto Millipore Filter
↓
Collapse Filter
↓
Carbon Coat Filter
↓
Transfer to Grid

C

Sample Air
↓
Onto Either Filter
↓
Ash Filter
↓
Resuspend in Water
↓
Onto Nuclepore Filter
↓
Carbon Coat Filter
↓
Transfer to Grid

D

Sample Air
↓
Onto Either Filter
↓
Ash Filter
↓
Resuspend in Water
↓
Centrifuge
↓
Decant Supernatant
↓
Drop onto Carbon Coated Grid

SPECIMEN **A·03**

FIGURE 4
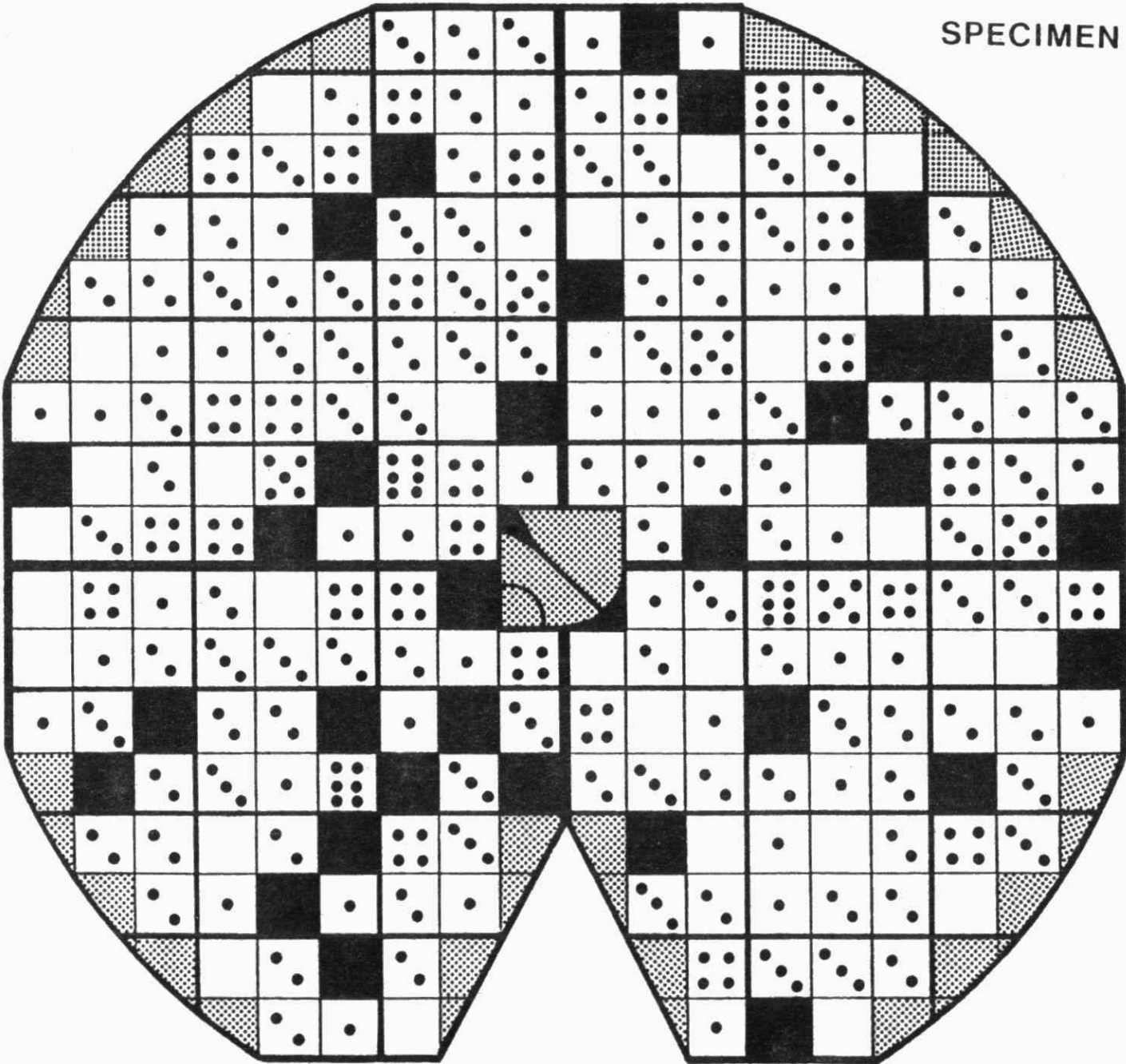
− 568 −

DETERMINATION OF METALS AND METAL COMPOUNDS
IN AIR AND RELATED SAMPLES

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ABSTRACT:

Work has been concentrated into 2 main areas, a study of Hg compounds in air and water and the direct introduction of air filter samples into an inductively coupled plasma and multitrace element analysis by emission spectrometry.

A. Mercury Compounds in Air and water

A study was made of the gas chromatographic - atomic absorption determination of dimethylmercury and methylmercury chloride in air. Parameters studied include: type of column packing, the influence of the composition of column, transfer line and injection valve surfaces, need for atomic absorption background correction and the efficiency of collection of the compounds. A procedure suitable for analysing building and street air was developed for the determination of dimethylmercury and methylmercury chloride (1). A Tennax column was employed and the temperature program was 150°C to 180°C at a rate at 20°C per minute. Organo mercury results of typical building and urban street air samples will be given. The detection limits for dimethylmercury and methylmercury chloride are 2 and 5 ng respectively. Preliminary results using a microwave plasma in emission as a detector for the gas chromatograph indicate that detection limits may be as much as 10 to 100 time better. A method is being developed for the determination of Hg compounds in water. An extraction of the compounds from samples acidified with HBr into a mixture of benzene and toluene was employed. The

latter is carefully evaporated to small volume and an aliquot injected into the gas chromatograph.

B. Direct Analysis of Air Filters

A Perkin-Elmer HGA 2100 was used to produce a vapour of air filters samples. The vapour was carried into the plasma in the sample carrier gas flow. This avoids the need for wet chemical sample treatment and the inherent problems due to losses and contamination. The Procedure involves taking a sample of a glass fiber filter paper using a paper punch. This sample is placed on a graphite platform. The platform is inserted into and centered in the graphite furnace. The furnace is purged with Ar and the flow rate through the furnace is adjusted to the optimum value of 400 ml/min. The sample is volatilized into the furnace. For refractory elements the Ar carrier gas is doped with 0.2% freon (helps volatillize elements as F or Cl compounds). Results have been obtained for Cu, Pb and V. Typical values for Pb and Cu are 4(9) ug/in^2 and 4(3) ug/in^2 respectively (average of several filters). The bracketed values were obtained by wet chemical methods. Values for V will be reported at the seminar.

MERCURY COMPOUNDS IN AIR

SCHROEDER [1] reviewed developments in the measurement of atmospheric mercury. He estimates that 83.5 tons of mercury was released by human activity in 1970. However, he found that few attempts had been made to separate mercury compounds in atmospheric samples. This is in spite of the well-known species dependency of mercury reactions in the environment and in toxicology. Dimethylmercury and methylmercury chloride are the organomercury compounds of interest in air.

BRAMAN and JOHNSON [2] developed a selective absorption type method for the determination of mercury species in air. For example 3% SE-30 on Chromosorb W 45-60 mesh absorbed mercury chloride and Chromosorb W treated with NaOH was used to retain methylmercury chloride. Each fraction was then separately analysed for mercury by DC discharge emission spectrometry. This procedure was presumably lengthy and was used only for the analysis of building air.

It is desirable to have a gas chromatographic method for the determination of organomercury species in air. Such a method should be applicable to samples of outside air. It may also be possible to use such a method, particularly if emission detection is used, to determine the organo species of more than one element simultaneously.

There have been many many reports on the gas chromatography of mercury compounds. However, to the present author's knowledge none exists describing the determination of the important mercury species in outside air samples. In addition there is little data on the optimum chromatographic conditions for the separation of dimethylmercury and methylmercury obtained from any sample type.

There have been 2 comprehensive reviews on the chromatography of organomercurials. FISHBEIN [3] in 1970 reviewed chromatographic and biological aspects of organomercurials, but little on the determination of dimethylmercury was found. In 1978 RODRIQUEZ-VAZQUE [4] reviewed the gas chromatography of organo-mercury II compounds. He makes the important point, which is emphasized in the work reported below, that metal surfaces cause decomposition of organomercurials.

There have been a number of relatively similar gas chromatographic papers starting with the classic paper by WESTOO [5], on the determination of mercury compounds in fish. These involve conversion of the mercury compounds to halides and then extraction into an organic solvent followed by a clean-up step to remove interfering fatty acids. A variety of gas chromatographic column packings have been recommended and often more than one type of column was necessary when dimethylmercury and methylmercury chloride were to be done. The present authors found that there was much confusion in the literature on the type

of column to be recommended for determination of dimethylmercury
and methylmercury. No gas chromatographic method for the
determination of these important compounds in air could be found.

EXPERIMENTAL

Equipment

Chromatography   A Fisher 2400 gas chromatograph was employed. It
was modified to contain no metal surfaces in contact with the gas
stream. A teflon insert was placed in the injector. Glas
columns 1 or 2 m long, 5 mm o.d. and 2 mm i.d. were employed.
The packing was Tenax or mixed Tenax - DEGS. A Tenax coating
using 250 ul of Silyl-8 was also studied. The recommended
chromatographic operating conditions are:

>   Gas (argon) - flow rate 100 ml/min
>
>   Injector Temperature - 200°C
>
>   Column Temperature Program - 150°-180°C/min.
>
>   A Teflon tube transfer line heated to
>
>   140°-180°C was employed

Atomic Absorption Detector.   A Perkin-Elmer 305B atomic
absorption unit equipped with a deuterium arc background
corrector was used. The radiation source was an mercury hollow
cathode lamp. An Omniscribe (Houston Instruments) single pen
chart recorder was employed at a chart speed of 0.5 cm.min. The

wavelength and slit widths were 253.6 nm and 0.7 nm respectively.

A quartz "T" tube atomizer was employed. This consisted of a quartz capillary (5 mm o.d. and 1 mm i.d.) pyrolyser (at 900°C) terminated by an open ended unheated 15 cm long by 1-15 cm i.d. quartz tube absorption cell (Fig 1). The addition of CuO to the pyrolyser as recommended by others was found to have little useful effect in this work.

Reagents

Dimethylmercury (Alfa Inorganics). A stock solution was prepared by weighing 0.05 ml into a 50 ml volumetric flask and diluting it with benzene. This was kept in a refrigerator at about 4°C. Working solutions were prepared fresh daily by dilution in benzene.

Methylmercury chloride (Alfa Inorganics). A sample weighing 100 mg was dissolved in 50 ml of benzene. The stock solution was kept in a refrigerator at about 4°C. Working solutions were prepared fresh daily in benzene.

Recommended Procedure

Air samples were collected on a short piece 14 x 0.8 cm length of glass chromatographic column packed with 20% Tenax and 80% 1 mm glass chips. Pumping speed was 2 l/min. Air was first

drawn through a Milipore membrane filter of 0.45 u pore size. After the air sample had been collected on the short column, the latter was connected to an argon supply and the mercury species desorbed by heating the column to about 200°C for 0.5 hour. An argon flow rate of 10 cm/min was used. The mercury compounds were collected in 0.1 ml of benzene in a micro impinger. The impinger solution was cooled in an ice salt water bath. [temp. about 6°C]. Appropriate aliquots of benzene solutions (10 or 20 ul) containing standards or sample were injected into the gas chromatograph. A temperature programs of 150°C to 180°C at 20°C per min. was used. A 1 m column was used with a carrier gas flow rate of 100 ml/min. Retention times for dimethylmercury and methylmercury chloride were 0.3 min and 1.8 min. respectively.

RESULTS AND DISCUSSION

Study of Column Packings

A variety of column lengths and packings were studied: Results are summarized in Table (1).

(1) 15% DEGS on Chromosorb W 80/100 mesh - 2 m length;

(2) 15% DEGS on Chromosorb W 80/100 mesh (1.5 m length); and Tenax 80/100 (0.5 m length)

(3) 15% DEGS on Chromosorb W 80/100 mesh (1 m length) and Tenax 80/100 mesh (1 m length);

(4) 15% DEGS on Chromosorb W 80/100 mesh (o.5 m length) and Tenax 80/100 mes
   length);

(5) Tenax 80/100 mesh (2 m length);

(6) Tenax 80/100 mesh treated with Silyl 8 (2 m length);

(7) Tenax 80/100 mesh (1 m length);

Table 1. Retention Times of Mercury Compounds

Column	Length m	Temperature conditions	Retention time (min)		
			Hg Vap	$(CH_3)_2Hg$	CH_3HgCl
1. Tenax 80-100 mesh	1	150-180° 20°/min 80-180° 20°/min	- 0.9-0.6	0.3 2	1.8 6
2. Tenax 80-100 mesh	2	80-180° 20°/min	0.5-0.	3.8-4	10-11
3. 75% Tenax 25%(15% DEGS) on Chromosorb W 80-100 mesh	1.5 0.5	70-100 4°/min for 15 minutes 100-180° 20°/min	1	4	16

DEGS. A packing of chromosorb W coated with 15% DEGS [6] is
usually used for methylmercury determinations. In our work the
best resolution between dimethylmercury and methylmercury
chloride was obtained using temperature programming of 60-140°C
at 20°/min e.g. (Fig.2). Under these conditions it was
impossible to separate the mercury vapour peak, if present, from
dimethylmercury. Changing of the carrier gas flow rate did not
improve the situation. This packing cannot be used when mercury
vapour is present with dimethylmerciry.

The sensitivities amounts give 0.044 absorbance; for this column are 8 and 40 ng of mercury in dimethylmercury and in methylmercury respectively.

TENAX and DEGS. When the column contained 50% DEGS on Chromosorb W and 50% of Tenax good separation between dimethylmercury and methylmercury was obtained under isothermal conditions (175°C) e.g. Fig.3. The separation of mercury vapor, if present, from dimethylmercury was difficult even at low temperatures. Sensitivities for the mercury compounds are similar to those obtained on a column containing only DEGS. The repeatability of the signal due to methylmercury chloride was poor.

As the amount of Tenax is increased the separation of mercury vapour, if present, and the two organo-mercury species improve. With a complex two step temperature programming (a) from 70 to 100°C with a rate of 4°/min and (b) from 100 to 180°C with a rate of 20°/min the resolution was very good [Fig. 4]. Retention times obtained with 15% DEGS (25%) - Tenax (75%) column are given in Table 1.

The amounts of mercury corresponding to 0.044 absorbance were 4, 5-6 ng and 33-35 ng for mercury vapour, dimethylmercury and methylmercury respectively. It was difficult to obtain good repeatability for methylmercury chloride.

TENAX. The separation of mercury vapour, when present, from the two organo-mercury species is best when a 100% Tenax packing was used with a 2 m column e.g. Fig. 5. [A simple one programming from 80-240°C with a rate of 20°C/min was used for Fig. 5]. The retention time for mercury vapor is the same as for the mixed DEGS-Tenax columns and for dimethylmercury and methylmercury chloride is greater with increasing amounts of Tenax.

The retuention time of methylmercury on Tenax columns was quite long. Because the separation of the mercury species was good it was found that a column as short as 1 m could in practice be used. (Fig. 6). The separation between dimethylmercury and methylmercury chloride is suitable for routine work using a programmed temperature from 150 to 180°C at 20°C/min. The retention time obtained with 1 and 2 m Tenax Columns are given in Table 1. An absorbance signal of 0.044 was obtained for 3.5 ng of Hg in dimethylmercury and 13-14 ng of Hg in methylmercurychloride.

After using a Tenax column for 2-3 weeks [the 2 m column is worse] some empty spaces (dead volume) are observed in the packing and the methylmercury chloride peak becomes irregular and repeatability decreases. To eliminate this problem the column was treated with Silyl-8 [Chromatograph Specialities Brockville Canada]. Ten 25 ul injections at 150°C were used. This eliminated the irregularities int he methylmercury peak. The sensitivities remained roughly the same.

Effect of Benzene

The chromatogram given in Figure 7 shows the methylmercury
and benzene peak obtained at 180°C. When programming is used the
benzene peak is very broad but is usually corrected by the
deuterium arc background corrector. At higher temperatures
benzene gives a very large absorbance which is not correctable.

A few times when even using the recommended procedure, a
small benzene peak was obtained. This may have been due to the
poor alignment of the atomizer in the optical beam or failure to
have the deuterium arc light beam and the hollow cathode lamp
light beam filling the same optical aperture.

Nature of Chromatographic Surfaces

In much of the early work in this study there was evidence
of losses or decomposition of the mercury compounds. This was in
spite of a concerted effort to prevent leaks and not to have
exposed metal surfaces [in a similar study with Pb[7] some metal
surfaces were found to decompose tetraalkyllead compounds]. It
became evident that on an all glass system [with a minimum of
Teflon connectors] optimized the results. Such a system, though
essential, is extremely difficult to use. When Teflon is used
the maximum column temperature should be reduced to 180°C. The
importance of using such a system cannot be over emphasized.

Collection Efficiency

A column packed as for collection of mercury species from the air was placed in the gas chromatograph. Gas was then passed through the column for 48 hours. The compounds were chromatographed using the conditions of the proposed procedure. Approximately 100% recovery was obtained for methylmercury chloride. Recovery for dimethylmercury was 98% as long as the collection was done at a temperature of 18°C or below. If the collection is to be done above this temperature then the collection tube should be cooled in a cold water bath during the collection-period. Peak areas were used in the above test because the chromatographic peaks (especially dimethylmercury were broader than those of the standards).

Repeatability

A number of injections of the two compounds (100 ng of mercury in methylmercury chloride and 8 ng of mercury in dimethylmercury were made and the chromatography done using the recommended conditions. The coefficients of variation obtained were 7% for methylmercury chloride and 6% for dimethylmercury.

Determination of Organo Mercury Species in Air.

During air sampling air was passed through the flow-meter only momentarily to test the flow rate. The average flow through the column trap was 2 l/min. In our work on street air it was necessary to sample for 10-30 hours. After the sampling was

finished the column was connected to the argon tank. The trap was heated using a heating tape to about 200°C for 0.5 hour and the compounds collected into 0.1 ml of benzene in the impinger cooled in ice. A slow flow of Ar (10ml/min) removed the mercury compounds from Tenax into the benzene. All the organic mercury compounds are retained by Tenax but only a part of the elemental mercury. For this reason mercury vapour was not included in the procedure. Figure 8a shows a typical chromatogram for a 20 ul injection of benzene, from collection of a real air sample, equivalent to 2 ng of Hg in dimethylmercury and 20 ng of Hg in methylmercury chloride. Table [2] is a compilation of results obtained during different days and for different lengths of collection. Detection limits are 2 ng and 5 ng for dimethylmercury and methylmercury chloride respectively.

Table 2 - Concentration of Mercury Species in Air

I. Room 322 Mining Building			
Sample	Volume of air (m^3)	Dimethyl mercury ($ngHg/m^3$)	Methylmercury chloride ($ngHg/m^3$)
1	15	0.5	30
2	16	tr	18
3	10	tr	14
4	10	tr	3
5	18	0.6	5
6	25	0.2	3
7	12	tr	3
8	12	1.3	2
II. Outside at 170 College St.			
1	30	0.4	tr
2	25	0.4	tr

CONCLUSIONS

A relatively simple method of the determination of organic mercury compounds by gas chromatography-atomic absorption spectrometry was described. Using a Tenax 2 m long column and programming from 80 to 180°C at 20°/min the separation of mercury vapour from dimethylmercury and methylmercury chloride is possible. In this case dimethylmercury peak appears at 120°C and the sensitivity of this compound is lower than at higher column temperatures. Therefore a short programming step from 150 to 180°C at 20°/min is recommended when the analysis is limited to organic mercury compounds. In both cases the background correction is important.

The direct connection of mercury collection trap to the gas chromatography resulted in broadened peak of dimethylmercury. This approach may be applicable with further research.

The work is now being continued to show the applicability of microwave plasma emission spectrometry as a detector for gas chromatography using the above methods.

DETERMINATION OF METHYLMERCURY IN WATER

Mercury levels in most natural waters are expected to be below 1 ug/l. Thus organomercury (most of which is expected to be methylmercury) will be some fraction of this amount. It is essential therefore to develop a very sensitive method for the determination of methylmercury in water. A solvent extraction procedure was developed.

EXPERIMENTAL

Equipment

Chromatographic and atomic absorption equipment is similar to that used for the determination of Hg compounds in air. A water bath kept at 85°C is used to evaporate solvents.

Chromatographic Conditions are:

Column   Glass 1.5 m x 5 mm o.d. packed with
                   Tenax 80-100 mesh

Column temperature 180°C

Injection temperature 200°C

Transfer line - Teflon tubing temperature 160-180°C

Reagents

Methylmercury chloride (Alfa Inorganics)

A sample weighing 100 mg was dissolved in 50 ml of benzene or toluene. The solution was kept at 4°C. Working solutions were prepared fresh daily in benzene or toluene. Baker analysed benzene and toluene were used.

Recommended Procedure

Samples were collected in acid washed (glass) bottles. They were stored at 40°C until extracted. (It is highly recommended where possible to do the extraction in the field.) Prior to analysis samples were allowed to warm to room temperature. One litre samples were used for extraction. Two g of KBr was added to the water in the sample bottle and the solution agitated to dissolve this material. (If there is difficulty in obtaining an emmulsion free separation 0.5 ml of Hf should be added at this stage.) A 5ml portion of a 3:1 mixture of benzene and toluene was added. The sample was vigorously shaken for 60 s and then allowed to settle for 20 m. All but 50 ml of the water layer was syphoned off and discarded. The reamining solutions were transfered to a 100 ml separatory flask and the layers carefully separated.

Standard solutions were extracted in an identical manner to the samples. One liter methylmercury standards containing 50, 100, 200 and 250 ng Hg were prepared and reagent blank was also

run. Four ml alignots of the organic extracts were placed in separate small glass vial impinger tubes. The solvent was evapourated to 0.1 ml in a water bath kept at 85°C. The final stage of the evaporation was watched very carefully to avoid haivng the evaporation go to dryness.

Twenty ul alignots were injected into the gas chromatograph. Chromatographic conditions as listed above were employed.

RESULTS AND DISCUSSION

Maximum Gas Chromatographic Injection Volume

It is desirable to use as large a portion of the 100 ul extract as possible in the gas chromatograph. Fifty and 100 ng of Hg as CH_3HgCl in different volumes of benzene (from 1 to 50 ul) were injected into the gas chromatograph. The recommended chromatographic conditions were employed. Results show that above 20 ul there is a slight decrease in sensitivity.

Effect of Salt Contents on the Extraction

A variety of salts were tested to determine their effect on the extraction efficiency. These were NaCl, KCl, KF, KBr and KI. Of these KBr showed a 30% and 100% improvement over chloride and iodide salts respectively.

The effect of different salt content on the extraction efficiency was investigated. The results are given in Table (3)

Table 3. Effects of NaBr on Extraction Efficiency (%)

Hg(in CH$_3$HgCl) ug/l	Salt Content (%)							
	0	0.1	0.2	0.5	1.0	2.0	5.0	10.0
10	88	94	97	96	83	88	78	62
20	90	43	98	97	92	87	80	63
30	92	95	98	97	93	86	79	60
40	91	94	97	96	93	85	80	60
50	92	96	98	97	92	86	80	60

As can be seen there is a rather narrow range of salt concentrations giving quantative recoveries. A concentration of 0.2% was chosen for the proposed procedure.

Effect of Sample pH on The Extraction

The pH of the sample solution was varied from 1 to 10 using HNO$_3$ and NH$_3$. The extraction procedure was then employed for each solution. No significant difference in extraction efficiency was noted over this pH range.

Effect of Shaking Time on the Extraction

A relatively large volume of water compared to solvent volume must be employed. For this reason it is important to investigate the length of time of shaking required to obtain a maximum recovery of the CH_3HgCl during the extraction.

Various shaking times from 30 s to 30 m were employed on solutions prepared as indicated in the proposed procedure. Suprisingly 20 to 60 s were found sufficient to give maximum recoveries.

Composition and Volume of Solvent

It was necessary to evaporate the solvent prior to injection into the gas chrometograph. Loss of Hg is, of course, possible during this operation. Losses of up to 40% were obtained using benzene. Thus various volumes of benzene mixed with toluene were investigated to seek an improved recovery. The proposed procedure was employed and the results are given in Table (4)

Table 4. Effect of Solvent Composition on Recovery of CH_3HgCl

Percentage of Tolnene	Recovery %	Evaporation Time hr.
0	66	3.5
5	72	4.2
10	88	4.5
20	96	4.8
25	98	5.0
50	90	5.5

The minimum volume of solvents which can be used for 1 l of water was found to be 5 ml. When the volume of water was increased the volume of solvent needed must be increased proportionately. A 3:1 mixture of benzene to toluene was chosen.

Analysis of Water by the Proposed Procedure

A number of river water samples were analysed by the proposed procedure. Results are given in Table (5).

Table 5. Determination of CH_3HgCl in Waters (ng/l).

Water	Sample	Result	Coefficient of variation
Humber	1	25	5
	2	25	5
	3	30	5
Creek (Toronto)	1	30	10
Creek (Mississauga)	1	45	10

These are the first results for methylmercury chloride in Toronto waters.

Because of the very low levels of CH_3HgCl in rain and lakes this compound has not been detected in such samples at this time of writing. However, as will be seen in the next section our work with the microwave plasma as a gas chromatography detector shows that better detection by at least 2 orders of magnetude have been achieved. Thus we will be doing the determination of CH_3HgCl in rain and lake water soon.

MICROWAVE PLASMA EMISSION DETECTOR FOR GAS CHROMATOGRAPHY

Work reported in the previous two sections of this report indicates that better detection limits are needed. The levels of mercury compounds in outside air and water are so low that work with the atomic absorption detector only allows analysis of relatively polluted samples. Thus we are developing a microwave plasma emission detector for use with the gas chromatography.

EXPERIMENTAL

Equipment

The microwave plasma was generated with a Microtron 200 Mark II 0-200 W power supply. The cavity was homemade and is our modification of the Beenakker (8) design (TM_{010}). Radiation was focussed by means of a 50 mm focal length by 25 mm diameter fused SiO_2 lens on the slit of a model H-20 Instruments S.A. Inc. monochromator. This monochrometer has 0.1 mm slits and a bandpass of 0.4 nm. The 253.7 nm Hg line was employed. A solar blind R166 (Hanamatsu TV) photomultiplier was used. Gas chromatography was similar to that reported in Section (1).

Reagents

These were similar to those used in Section (1).

Procedure

This was similar to the procedure in Section (1).

RESULTS AND DISCUSSION

Optimization of Experimental Conditions

The retention time for CH_3HgCl was 3.5 minutes. The system was optimized for the following parameters.

(1) flow rate

(2) forward microwave power

(3) spatial variations

The flow rate will effect the plasma and the chromatography system. The signal is optimal for a flow rate of a 130 $ml.min^{-1}$ Therefore the emission signal is maximum at a flow rate of a 130 ml min^{-1}.

The efficiency of the column, as measured by the number of theoretical plates, was calculated using the following squation:

$$N = 5.54 \ \left(\frac{tr}{W^{1}/_2}\right)^2$$

where tr = retention time and $W^{1}/_2$ equals the peak width at half height. The column efficiency is at a maximum at low flow rates. However, the efficiency at the flow rate where the emission maximum occurs is not significantly lower than the maximum efficiency.

The microwave power is optimized at a level of 70 watts. The system was previously found to be at an optimum in terms of spatial variations, when the centre of the plasma was focussed on the entrance slit.

Detection Limits

Although the detection limit has not been experimentally established, it can be estimated to be between 10 and 30 pg. This detection limit is much better than the detection limit for G.G./A.A.S. which is 5 ng for methylmercuric chloride.

Dynamic Range

Linearity of response has been observed over a limited range (0-2 ng). However it is expected that the dynamic range will extend over several orders of magnitude.

Reproducibility

The reproducibility for CH_3HgCl is about $\pm 3\%$ for a 1.25 ng sample (peak height).

Conclusion

The G.G./M.I.P. has great potential as a detector for CH_3HgCl in air and water samples. It has good sensitivity and high selectivity.

TRACE ELEMENTS IN AIR FILTERS BY DIRECT INJECTION OF FILTER AEROSOL INTO ICP

An air filter sample is analysed by placing a punched out circle of the Filter into a graphite furnace which is connected to the base of the ICP torch. The furnace is rapidly heated and the aerosol thus produced is swept into the plasma.

EXPERIMENTAL

Equipment

An Applied Research Laboratories 34000 Quantometer which is supplied with a PDP 11 - computer (Digital) was used.

A Perkin Elmer HGA 2100 was used to produce the sample aerosol (vapour). The furnace housing was very easily adaptable to interface with the torch of the plasma through a Quartz tube friction fitted to a graphite ring in one end of the furnace.

The two components are interfaced with a J. tube. The complete interfacing module consists of

(1) "j"-Quartz transfer tube with valve and side arm.

(2) Pressure reducing system (concentric Meinhard nebulizer).

(3) Flowmeters with high precision valve controls.

A photograph of the equipment is shown in Fig.9. A schematic of
the system is given in Fig.10.

ICP Operating Conditions are:

Incident power	1300 W
Reflected power	<10 W
Coolant gas pressure	50 PSI
Plasma gas pressure	35 PSI
Carrier gas flow rate	0.5 Litres min^{-1}
Sample aerosol flow rate	0.4 Litres min^{-1}

Furnace operating conditions are:

Drying time	0
Ashing time	20 sec.
Ramp.	10 sec.
Temp.	200°C - Pb
	650°C - Cu
Atomization/volatilization time	8 Cu
Ramp.	0
Temp.	>2800°C

Reagents

All reagents were Reagent Grade ACS. Stock metal standards
were prepared from spec pure metals dissolved in a minimum amount
of nitric acid. Part per billion working solutions were prepared
fresh daily.

Procedure

A punch is used to obtain a sample of the glass fibre filter paper. The sample is then pressed into the slot on a graphite boat. The aluminium cap is removed and the boat slid into the furnace tube. The boat is centered using a centering tool. The bypass valve is then opened and the furnace purged with Argon. The flow rate is set to the operating level of 400 ml Min^{-1}. The furnace program is then actuated. The peaks are recorded on the chart recorder. Calibration standards are prepared by pipetting ul volumes of standard solutions on a punched out piece of blank glass fibre filter paper. Standards are run in the same way as the samples.

RESULTS AND DISCUSSION

Oxygen Problem

The argon plasma is sensitive to the presence of oxygen in the system and will extinguish easily if a sudden surge of air or argon/air mixture is introduced into its central channel. A bypass valve on the transfer tube is designed to iosolate the furnace from the torch during insertion of a solid sample into the furnace.

Argon sample uptake flow rate

There are 3 gas flows to the plasma torch. The sample aerosol (vapour) from the furnace is injected into the central

axial channel. The optimum flow for this is between 0.7-1.0 live min^{-1}. In order to maintain a stabilized plasma, this argon flow is split up into two fractions, one fraction is fed directly and continuously into the central channel and the second fraction is routed through the furnace. Experiments have been performed to optimize this splitting for both plasma stability and signal response.

Results

Air filters were run by the proposed procedure for Pb and Cu and the results given in Table (6).

Table 6. Results: (ICP) Values for Pb, Cu and V in Air Filters

SAMPLE #	SOLID SAMPLING $\mu g. \ m^{-3}$			CONVENTIONAL DIGESTION $\mu g. \ m^{-3}$		
	Pb	V	Cu	Pb	V	Cu
1	1.3	3.3	0.8	1.6	5.7	0.8
2	2.6	9.6	1.6	4.3	12.0	1.2
3	2.4	4.7	0.8	3.0	4.8	0.4
4	3.5	4.5	1.2	4.9	5.5	1.2
5	4.3	7.3	2.4	8.3	8.8	1.6

The results were compared with those obtained by a conventional digetion. Acceptable agreement was obtained for Cu. The results at this stage are not as good for Pb and V.

REFERENCES

1. W.H. SCHROEDER, Canadian Research, May 3B, (1981).

2. R.S. BRAMAN and D.L. JOHNSON, Environ. Sci. and Technol. 8, 996, (1974).

3. L. FISHBEIN, Chromatog. Rev. 13 83, (1970).

4. J.A. RODRIQUEZ-VAZQUEZ, Talanta 25, 299, (1978).

5. G. WESTOO, Chem. Scand. 20, 2131, (1966).

6. G.L. BAUGHMAN, H.H. CARTER, N.L. WOLF and R.G. ZEPP, J. Chromatog. 76, 1971, (1973).

7. B. RADZIUK, Y. THOMASEN, J.C. VAN LOON and Y.K. CHAU, Anal. Chim. Acta 105, 255, (1979).

8. C.I.M. BEENAKKER, Spectrochim. Acta 31B, 483, (1972).

LIST OF FIGURES

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Fig.2 Chromatogram of mercury compounds separated on a 2 m
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               Column temperature - 60-140°C programmed at 20°/min
           55 ng of mercury in dimethylmercury
          750 ng of mercury in methylmercury chloride.

Fig.3 Chromatogram of mercury compounds separated on a 2 m
       column packed with 15% DEGS on Chromosorb W 80-100 mesh
       (50%) and Tenax 80-100 mesh (50%).
               Injector temperature - 200°C
               Column temperature - 160°C
           40 ng of mercury in dimethylmercury
          300 ng of mercury in methylmercury chloride.

Fig.4 Chromatogram of mercury compounds separated on a 2 m
       column packed with 15% DEGS on Chromosorb W 80-100 mesh
       (25%) and Tenax 80-100 mesh (75%).
               Injector temperature - 200°C
               Column temperature: 70-100°C programmed at 4°/min
               and from 100-180°C programmed at 20°/min
           20 ng of mercury vapour
           40 ng of mercury in dimethylmercury
          500 ng of mercury in methylmercury chloride.

Fig.5 Chromatogram of mercury compounds separated on a 2 m
       column packed with Tenax 80-100 mesh.
               Injector temperature - 200°C
               Column temperature - 80-240°C programmed at 20°/min
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           10 ng of mercury in dimethylmercury
          400 ng of mercury in methylmercury chloride.

Fig.6 Chromatogram of mercury compounds separated on a 1 m
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          300 ng of mercury in methylmercury chloride.

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Fig.8   Chromatogram of air sample on 1 m Tenax column. Injector
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         B.3.2 m^3 of air with additions of mercury standards
            5 ng of mercury in dimethylmercury and
           25 ng of mercury in methylmercury chloride

Fig.9   Photograph of Furnace ICP Equipment.

Fig.10 Schematic of Furnace ICP system.
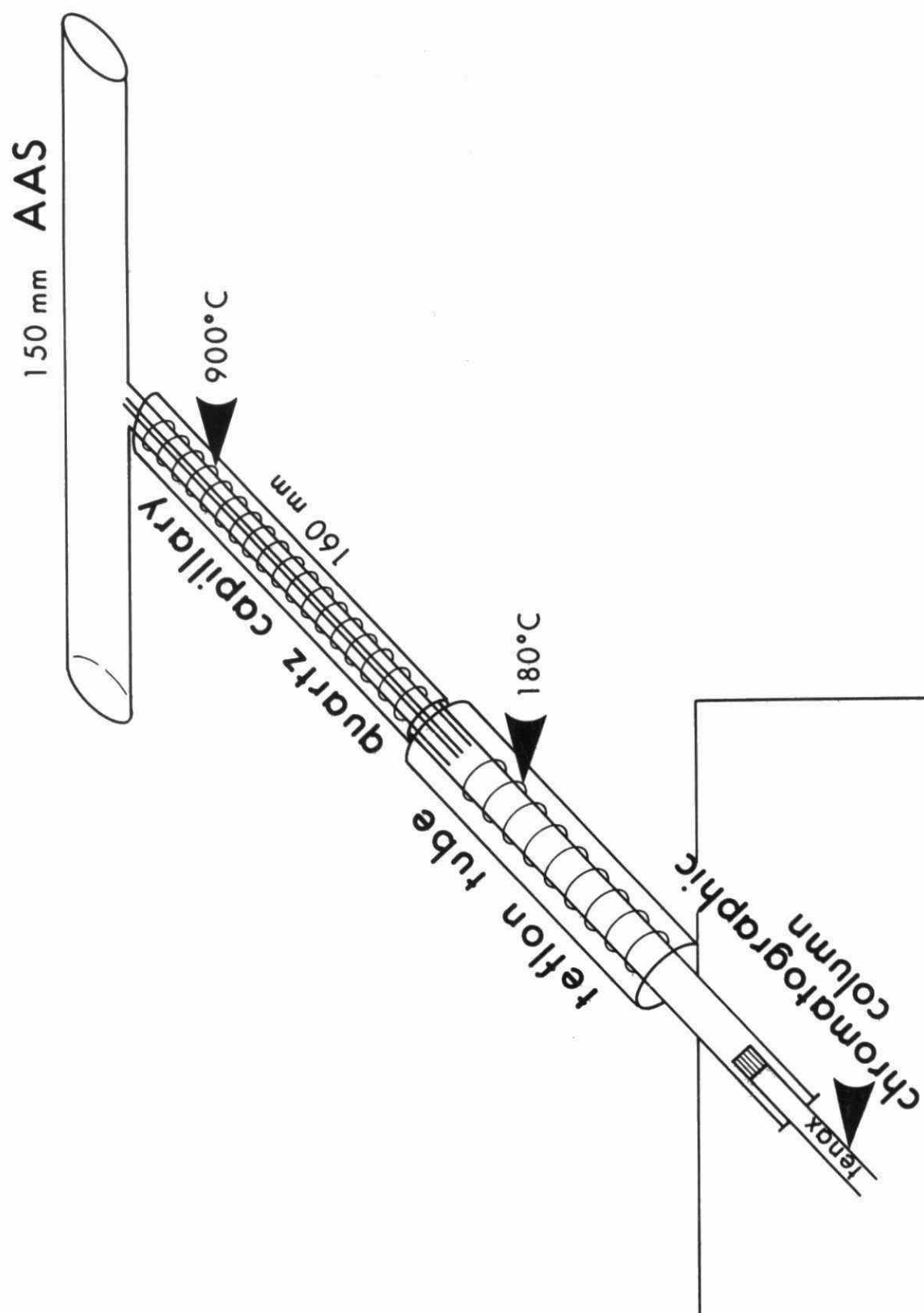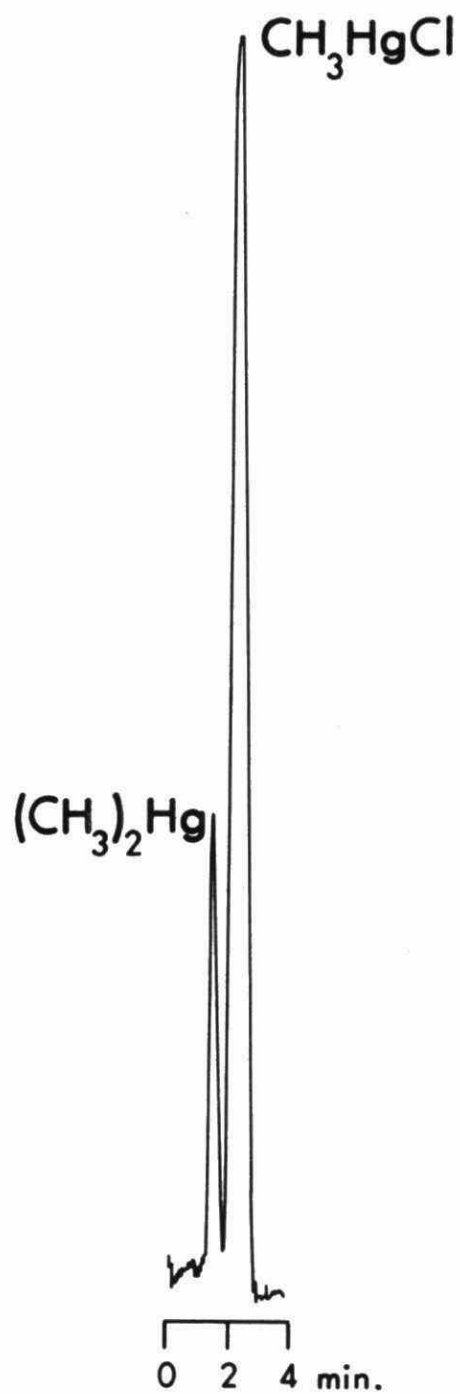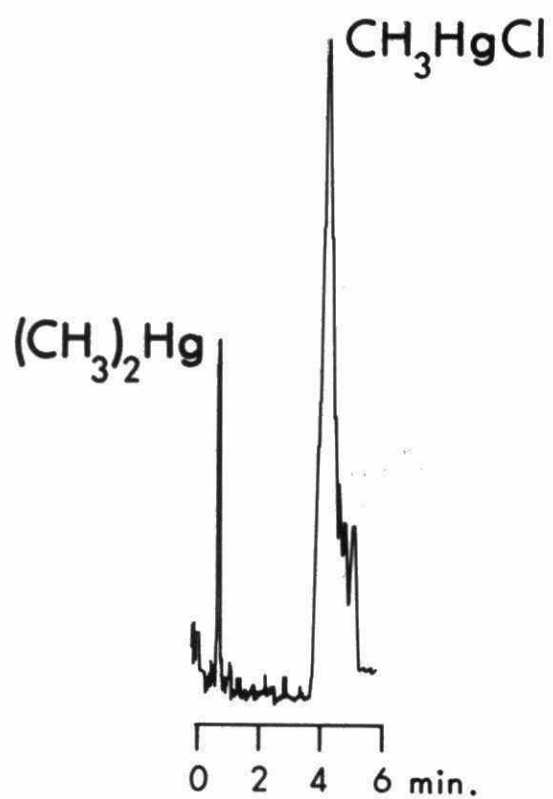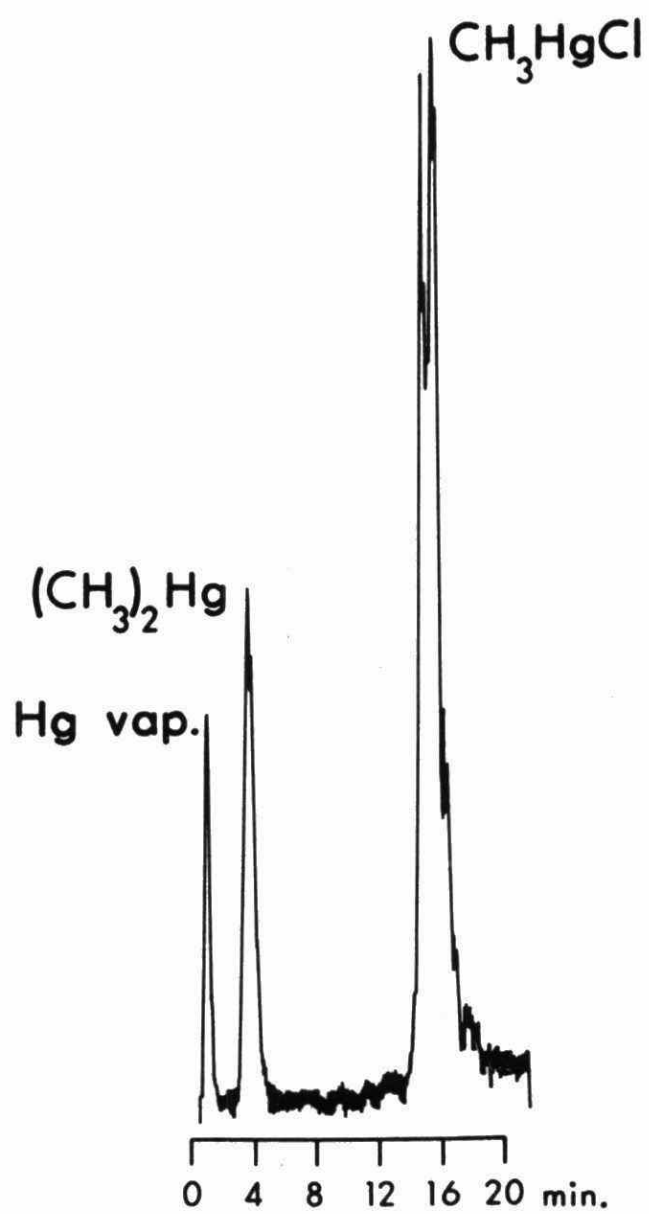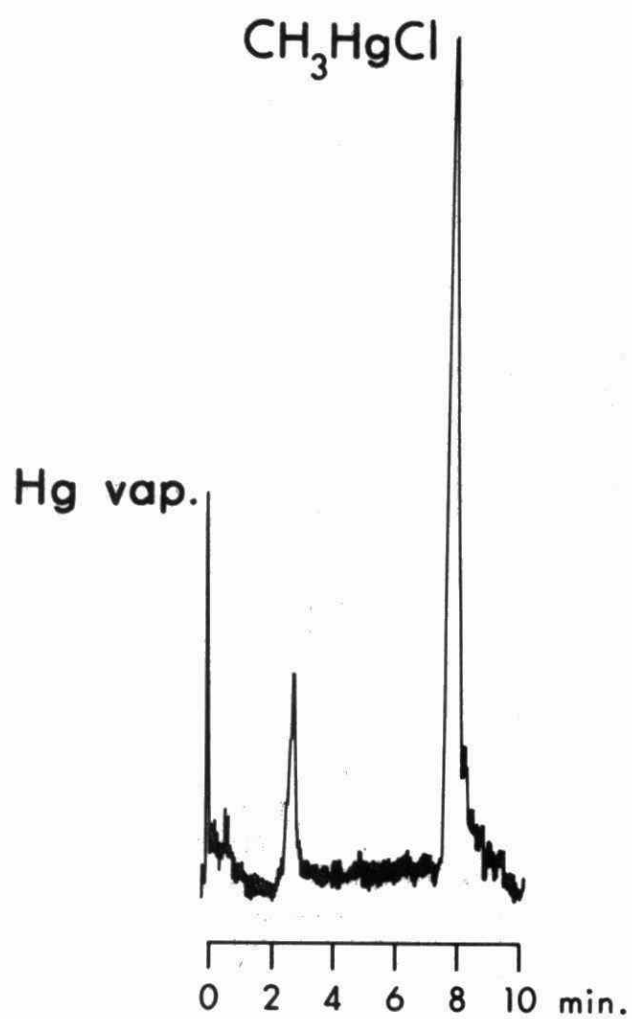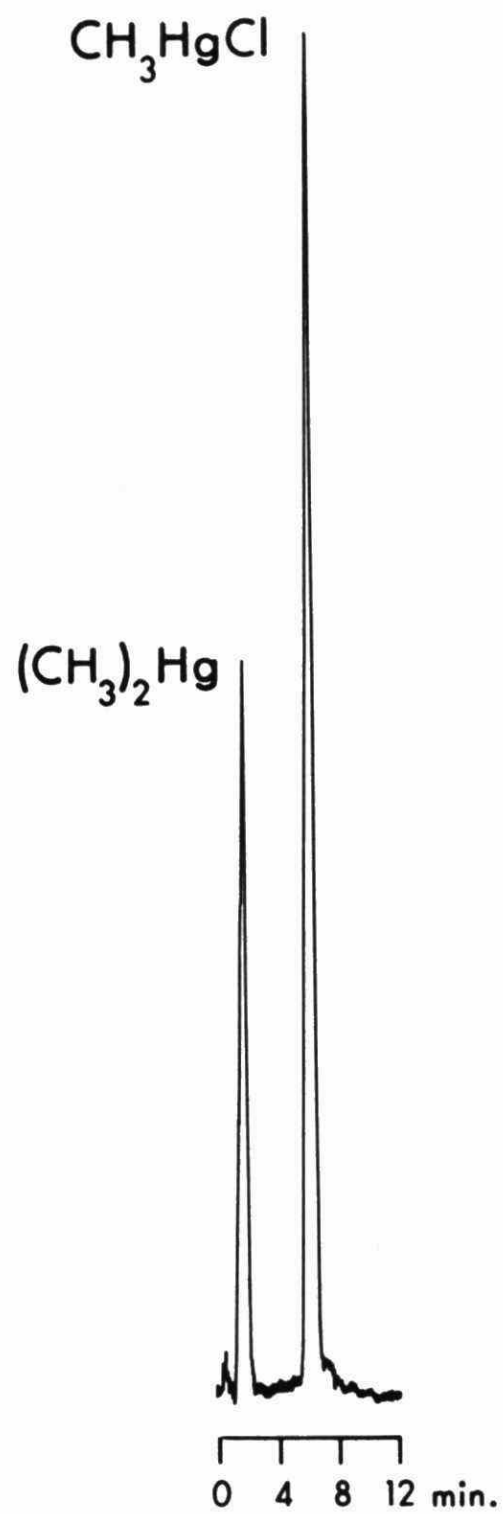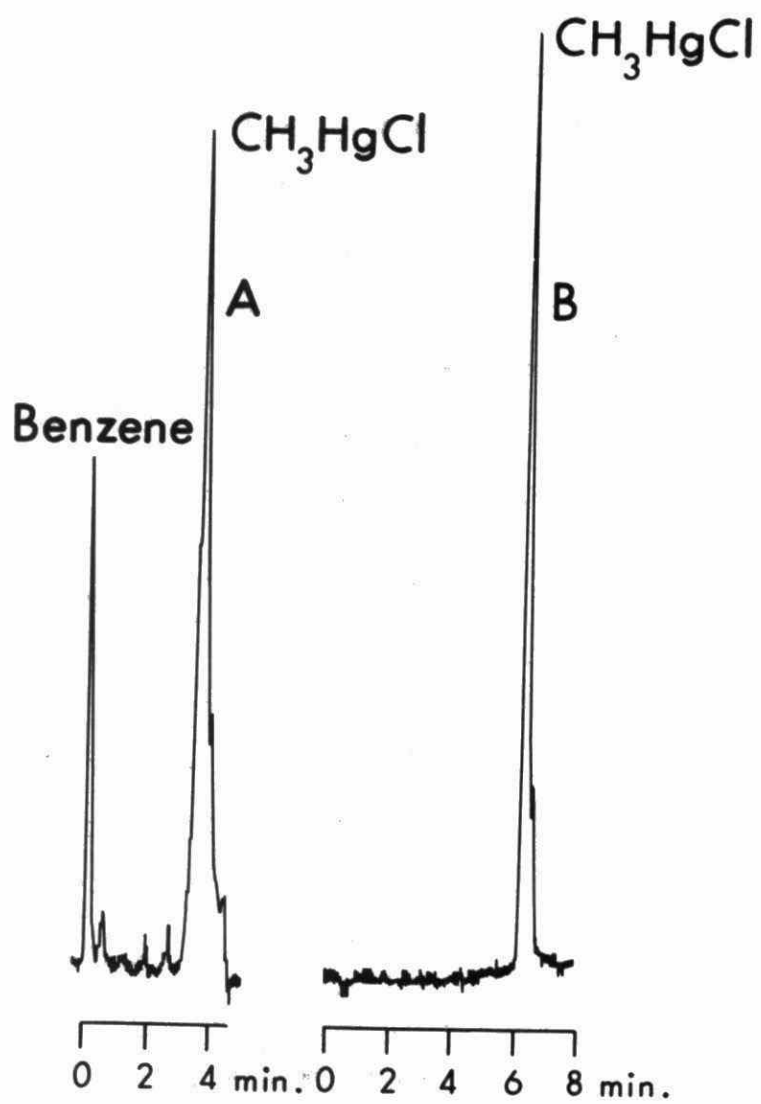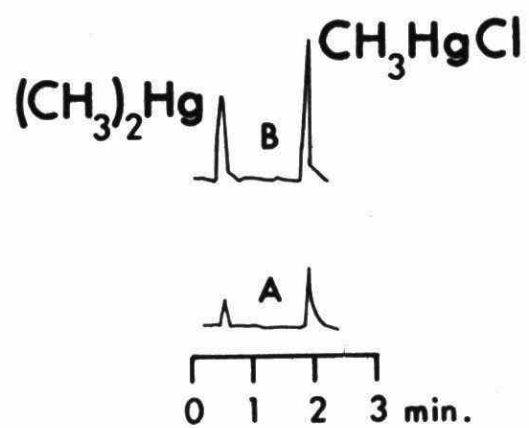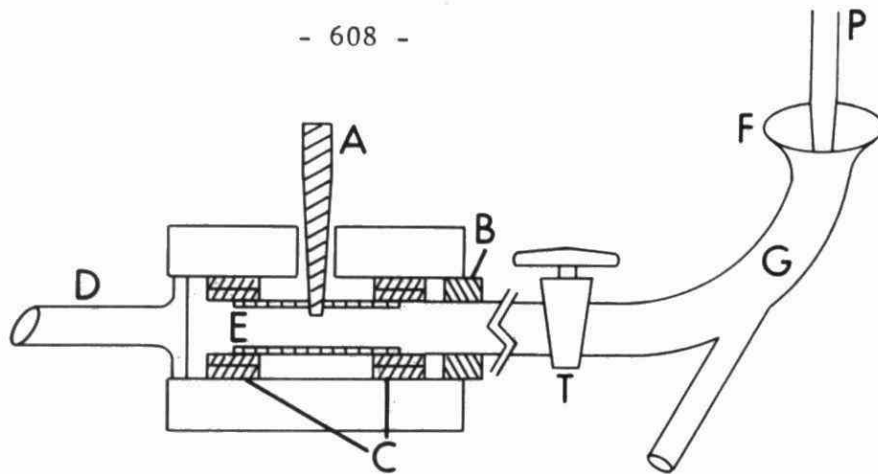
A Graphite plug
B Graphite bushing
C Graphite cones
D Aluminium end cap
E Furnace tube
F Ball & Socket joint
G Transfer tube
T Tap
P Plasma torch

A   Furnace power supply
GTF Graphite tube furnace
T_1 Tap to plasma torch
T_2 Tap to furnace
P   Plasma torch
F_1 Flowmeter valve sample aerosol
F_2 Flowmeter valve carrier gas
N   Concentric Nebulizer
B   Auxiliary bottled gas argon supply
C   Aluminium end cap
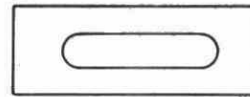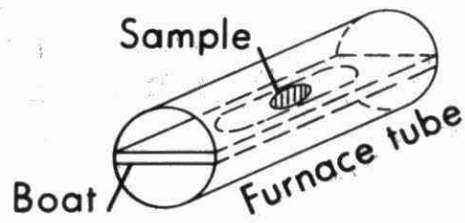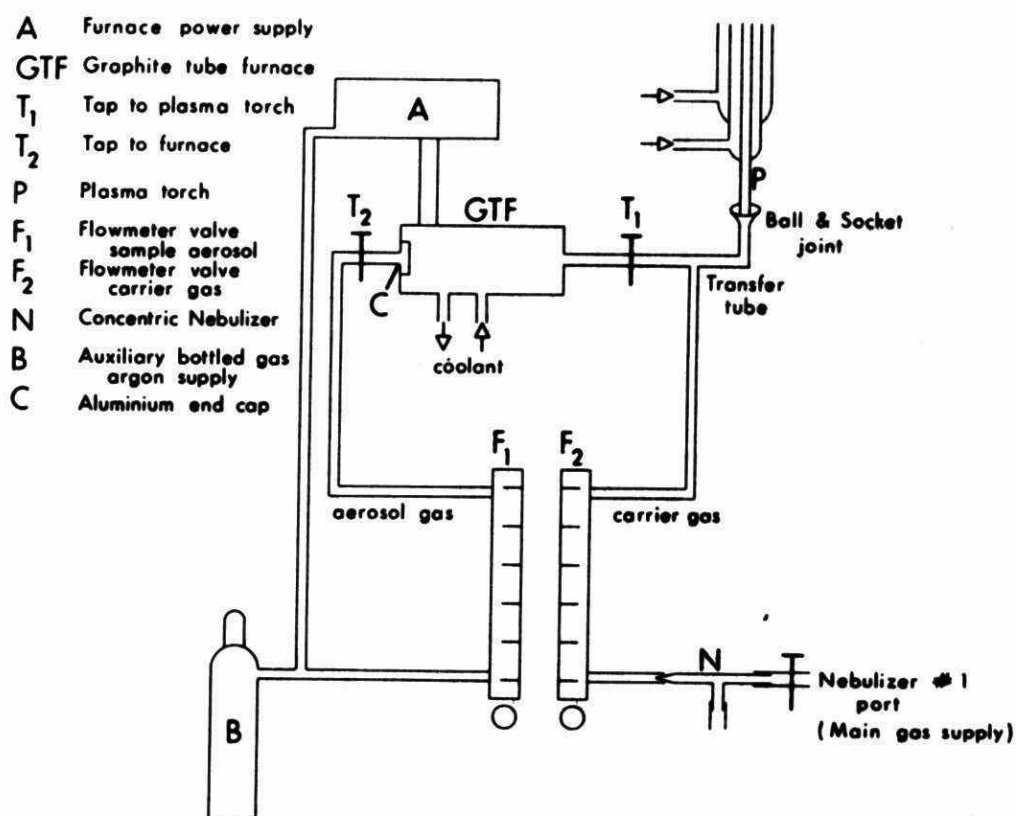
HEALTH EFFECTS OF AIR POLLUTION

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The health effects of air pollution, particularly at low levels, are still somewhat uncertain. Most studies to date have utilized imprecise assessments of personal exposure to air pollution because of their reliance on pollution data obtained from fixed air pollution stations monitoring outside air. Because individuals can live some distance from such stations and because they spend up to 90% of their time indoors the need for better estimates of exposure has arisen. A personal portable multi-pollutant sampler has been designed at The Gage Research Institute and measures concentrations of nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and suspended particular matter (SPM). Another problem with previous studies has been the use of severe symptoms or signs as measures of health effects while ignoring minor fluctuations in symptoms and pulmonary function. In an attempt to remedy these deficiencies a study was designed to measure the health effects of air pollution.

Thirty asthmatics and fourteen healthy non-asthmatics were involved over the period January 1981 to July 1982. Each subject was visited at his home on up to twenty days both in the morning and afternoon, contributing a total of approximately 500 person-days. On each day pulmonary function was assessed in the morning. Measurements included forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and maximum mid-expiratory flow rate ($FEF_{25\%-75\%VC}$).

A personal multi-pollutant sampler was then activated and carried by the individual for the rest of the day up until about 4 P.M. at which time it was shut off and pulmonary function again measured. The two visits on each day permitted not only as estimate of the subject's pulmonary function on a given day but also the change over the day. These pulmonary function tests were administered according to standard procedures. At each session at least 3 maximum expiratory manoeuvres were performed by the subject until 2 spirograms were obtained which were reproducible to within 5% for both flow and volume. The Vitalograph was calibrated monthly for volume using a one litre syringe and time.

Levels of Pollutant Concentration:

The frequency distribution for NO_2 and SO_2 obtained using the sampler carried by the subject (PERSONAL) and by the sampler situated at the Ministry site (GAGE MOE) are shown in Figures 1 to 2. The total number of sampling days is 379.

Within Individual Analysis:

The potential effect of the exposure to NO_2, and SO_2 on pulmonary function was analyzed by means of different strategies listed in Table 1.

A.   Relationship Between Daily Change in Pulmonary Function and Daily Pollutant Concentrations.

The first strategy was concerned with an assessment of the relationship between the change in pulmonary function over the day and the corresponding pollutant concentration on that day. This was accomplished by simply calculating for each individual the correlation coefficient and slope of the relationship between daily pollutant concentrations and the corresponding day's change in pulmonary

function. For each individual, 4 slopes were calculated, one between each of the two pulmonary function changes and each of the two pollutants measured. For each individual these slopes were based on the same number of days on which complete information was collected on the two pulmonary function changes and the two pollutants. However the number of days on which a slope is calculated will vary among the individuals.

The change in pulmonary function was measured by substracting the afternoon value from the morning value. Therefore this difference will be positive if the pulmonary function decreased over the day. Therefore if the drop in $FEF_{25\%-75\%VC}$ was larger for an individual on those days for which the SO_2 concentration was high, then the slope and correlation coefficient between the daily change in $FEF_{25\%-75\%VC}$ and the corresponding day's SO_2 concentration would be positive. An example is shown for one of the subjects in Figure 3. This subject had complete information on 18 days and the slope between the daily change in $FEF_{25\%-75\%VC}$ and the corresponding day's SO_2 concentration was 0.42. This can be interpreted to mean that an increase in SO_2 concentration of one part per billion will be associated with an additional drop in $FEF_{25\%-75\%VC}$ of 420 ml/sec. The correlation coefficient for these 18 data points is 0.60 (p<.01). For each asthmatic and each non-asthmatic a slope was calculated between the daily change in $FEF_{25\%-75\%VC}$ and the daily SO_2 concentrations. If there were no relationship between the daily change in $FEF_{25\%-75\%VC}$ and SO_2 then one would expect half of the slopes to be positive and half to be negative. If, on the other hand, a deleterious relationship exists then one would expect an excess of positive

slopes. This hypothesis was tested using the sign test. The test of whether the mean and median of these slopes was different from zero was conducted using the paired t-test and the Wilcoxon signed rank test respectively. These analyses were conducted for both pulmonary functions and both pollutants. The results are shown in Tables 2 and 3.

Only the slope between the daily change in $FEF_{25\%-75\%VC}$ and the daily SO_2 concentration was found to be statistically significant. The median slope is 10.8. That is, for each increase of one part per billion in SO_2 and additional drop of 10.8 ml/sec in $FEF_{25\%-75\%VC}$ could be expected.

B. Relationship Between Daily Mean Pulmonary Function and Daily Pollutant Concentrations.

The statistical analysis in strategy A was concerned with the question of whether the effect of exposure to higher concentrations of the pollutants NO_2, and SO_2 would be manifested in a drop in pulmonary function on that same day. However, only one of the relationships, namely that between SO_2 and $FEF_{25\%-75\%VC}$ was found to be significant. One reason for this finding might be that the effect of higher pollutant concentrations on pulmonary function might be more gradual. Therefore those days on which the pollutant concentration was high may be associated not with a drop in pulmonary function but with a lower pulmonary function generally over the entire day. Under these circumstances we might expect a negative correlation between pulmonary function and pollutant concentration over the days monitored for each subject. Therefore in the same manner as was outlined in strategy A, a correlation coefficient and slope was estimated for each subject. However as the dependent variable, the daily mean pulmonary function was used instead of the daily difference in pulmonary function. The

results of these analyses are shown in Tables 4 and 5. As before the sign test was used to test whether there was a significant increase in the proportion of negative slopes. The paired t-test and Wilcoxon signed rank test were used to test whether the mean slope and median slope were significantly negative. No relationships were statistically significant.

Further Within-Individual Analyses:

All of the analyses were repeated with the pollutant concentration being replaced by the logarithm of the pollutant concentration. Because the SO_2 concentration could be as low as zero, a constant value of 1 was added to the SO_2 concentration before using the log transformation. Because only the results obtained between the daily mean $FEF_{25\%-75\%VC}$ and the log NO_2 and log SO_2 concentrations were significant they will be discussed at some length. The results that proved significant were those that used the NO_2 and SO_2 concentrations measured at the ministry site with the Gage sampler (GAGE MOE) and not using the sampler carried by the subject (PERSONAL). Furthermore the following discussion will emphasize the importance of using an analysis that measures the relationship within individuals and not across individuals.

The correlation coefficient between the daily mean $FEF_{25\%-75\%VC}$ and the corresponding log NO_2 concentration was very low, $r=-0.03$. The correlation between $FEF_{25\%-75\%VC}$ and the log SO_2 concentration was also very low, $r=0.01$. Both of these coefficients were based on a total of 379 person-days pooled across 41 subjects.

This analysis was repeated using the individual as the sampling unit and correlating the mean daily $FEF_{25\%-75\%VC}$ of an individual with

his mean log NO_2 and mean log SO_2 concentration averaged over the same number of days. This analysis was based on the same 41 subjects and again the correlation coefficients were very low, being -0.01 for the relationship between an individual's mean $FEF_{25\%-75\%VC}$ and mean log NO_2 and r = C.17 for the relationship between the individual mean $FEF_{25\%-75\%VC}$ and mean log SO_2. However as before a slope was calculated for each individual for the relationship between the daily mean $FEF_{25\%-75\%VC}$ and the corresponding day's log NO_2 and log SO_2 concentration. A scattergram of the relationship between $FEF_{25\%-75\%VC}$ and log NO_2 for one particular subject is shown in Figure 4. As seen for this subject, there is a strong negative relationship between the daily mean $FEF_{25\%-75\%VC}$ and the daily mean log NO_2 concentration. The slope was -0.64 ml/sec/log ppb and the correlation coefficient was -0.77 ($p < 0.01$). There were N = 39 individuals that had at least two complete days of information and for whom slopes could be calculated. If there were no relationship between daily mean $FEF_{25\%-75\%VC}$ and daily log NO_2 then one would expect half of the slopes to be negative and half positive. However as shown in Figures 5 and 6, the mean slope for $FEF_{25\%-75\%VC}$ and log NO_2 was significantly negative, -86 ml/sec/log ppb ($p < .05$). Similarly the mean slope between $FEF_{25\%-75\%VC}$ and log SO_2 was also significantly negative, -71 ml/sec/log ppb ($p < .05$). Although these two results were not dramatic, the p value being only less than 0.05, they do illustrate that quite divergent results can be obtained by analyzing results within individuals rather than either pooling results across person-days or analyzing across individuals the relationship between individual mean $FEF_{25\%-75\%VC}$ and the corresponding mean pollutant concentration.

Between Individual Analysis:

As indicated in Table 1 two major types of statistical analyses were used to assess the relationships between the exposure variables and the response variables. The first type reported above analyzed changes within individuals. The second type analyzed relationships across individuals. This analysis is designed to answer the question - do individuals who are exposed to high pollutant concentrations exhibit larger response.

One reason that this type of analysis might improve the chance of detecting a relationship is that the range of pollutant concentrations to which different individuals are exposed might be larger than that expected for a single individual. If this were so then the reliability of slopes for the between individual analysis could be expected to be higher than that for the within individual analysis. To test this possibility an analysis of variance of the two pollutant variables was performed to determine the relative sizes of the variance between individuals and the variance within individuals and found the variance within individuals to be much larger than that between individuals. Therefore if the between individual analysis is to prove more informative it will not be due to the increased range of pollutant concentrations faced by different individuals. In fact the larger variance of the pollutant concentrations within individuals probably reflects the measurement error of the monitoring network which can be decreased by using mean values of individuals rather than the single measurements. Therefore using mean values of individuals as the basic sampling unit for analysis might improve the analysis because of the increased reliability of such mean values relative to the single observations that comprise it.

C.   Relationship Between An Individual's Mean Change in Pulmonary
Function and His Overall Mean Pollutant Concentrations.

For each individual the mean concentration of NO_2 and SO_2 was
calculated over the number of days in the study that the subject had
complete data on both pollutant concentration and pulmonary function.
Over the same sequence of days, the mean daily change in pulmonary
function was calculated, that is, the mean of the morning value minus
the mean of the afternoon value. As in analysis A if the pollutant
has a deleterious effect on pulmonary function then we would expect
individuals exposed to high pollutant concentrations to have a larger
positive difference in pulmonary function over the day.   Only for NO_2
is there any indication of an effect (6).   The correlation coefficient
between the mean NO_2 concentration and the mean change in FEV_1 is 0.26
(p=.08) and with the mean change in $FEF_{25\%-75\%VC}$ is 0.33 (p=.03).
However in both cases the statistical significance is substantially
reduced when percentage change in FEV_1 and $FEF_{25\%-75\%VC}$ are used
instead of linear change, the correlation coefficients being 0.17
(p=.27) for FEV_1 and 0.22 (p=.16) for $FEF_{25\%-75\%VC}$.

Repeating this analysis for the asthmatics and non-asthmatics
separately revealed interesting differences (7).   The relationship
between mean NO_2 concentration and mean change in FEV_1 was much
stronger in the group of non-asthmatics, the correlation coefficient
being 0.61 (p=.02) whereas it was only 0.19 (p=.31) for the
asthmatics.

D.   Relationship Between An Individual's Overall Mean Pulmonary Function and His Overall Mean Pollutant Concentrations.

As in analysis D, the mean concentration of NO_2 and SO_2 was calculated for each individual over those days on which complete pollutant and pulmonary function data was collected. For each individual over the same sequence of days the mean pulmonary function measurement was calculated. If the pollutant has a deleterious effect on pulmonary function then one would expect an inverse relationship between mean pollutant concentration and mean pulmonary function. The results are reported in Tables 8 and 9.

The positive correlation between NO_2 and FEV_1 is similar for both asthmatics (r=.39) and non-asthmatics (r=.35) although only significant for asthmatics.

The positive correlation of 0.45 between NO_2 and $FEF_{25\%-75\%VC}$ is only significant for the asthmatics. Only for asthmatics is the correlation coefficient between SO_2 and FEV_1 (r=0.40) significant. These results illustrate the serious problems of interpretation that can be encountered in the analysis of cross-sectional data which is what analysis E actually is. We are attempting to estimate relationships across individuals by taking single measures for each variable in the relationship. Again we do not interpret these results to mean that exposure to high concentrations of NO_2 will improve a patient's FEV_1 but rather that the less healthy asthmatic with the lower FEV_1 will possibly modify his environment so as to reduce the NO_2 concentration to which he is exposed. The same interpretation could be applied to the other significant and positive relationships found in Table 8 and 9.

SUMMARY

1. The choice of scale used for the pollutant concentration can affect the relationship between pollutant and lung function.

2. Pollutant concentration may be related to the average daily pulmonary function or to the change in pulmonary function over the day.

3. The choice of sampling unit can be very important. Relationships between pollutant levels and lung function within individuals can be masked by analyses across individuals or by analyses that pool the data from all of the individuals.

TABLE 1. STRATEGIES OF ANALYSIS

1. WITHIN INDIVIDUAL ANALYSIS

    (A) Relationship between daily change in pulmonary
         function and daily pollutant concentration

    (B) Relationship between daily mean level of
         pulmonary function and daily pollutant concentration.

2. BETWEEN INDIVIDUAL ANALYSIS

    (C) Relationship between an individual's mean change in
         pulmonary function and his overall mean pollutant
         concentration

    (D) Relationship between an individual's overall mean
         pulmonary function and his overall mean pollutant
         concentration.

TABLE 2. RELATIONSHIP BETWEEN DAILY CHANGE IN FEV_1, $FEF_{25\%-75\%VC}$ AND DAILY NO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec)
Number of individuals	43	43
Proportion of positive slopes	0.51	0.51
Mean slope*	1.30	0.03
Median slope	0.05	1.63

*Slopes are reported as changes in pulmonary function (ml or ml/sec) per part per billion of pollutant.

TABLE 3. RELATIONSHIP BETWEEN DAILY CHANGE IN FEV_1, $FEF_{25\%-75\%VC}$ AND DAILY SO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec
Number of individuals	43	43
Proportion of positive slopes*	0.51	0.63
Mean slope	-4.00	31.40
Median slope	0.14	10.8**

*Slopes are reported as changes in pulmonary function (ml or ml/sec) per part per billion of pollutant.

**$p < 0.05$

TABLE 4. RELATIONSHIP BETWEEN DAILY MEAN PULMONARY
FUNCTION AND DAILY NO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec)
Number of individuals	43	43
Proportion of negative slopes	0.42	0.52
Mean slope*	2.20	3.60
Median slope	0.80	-0.12

*Slopes are reported as change in daily mean pulmonary
function (ml or ml/sec) per part per billion of
pollutant.

TABLE 5. RELATIONSHIP BETWEEN DAILY MEAN PULMONARY
FUNCTION AND DAILY SO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec)
Number of individuals	43	43
Proportion of negative slopes	0.47	0.47
Mean slope*	6.00	-7.80
Median slope	1.63	0.90

*Slopes are reported as changes in daily mean pulmonary
function (ml or ml/sec) per part per billion of
pollutant.

TABLE 6. CORRELATION COEFFICIENT BETWEEN MEAN
POLLUTANT EXPOSURE AND MEAN CHANGE
IN PULMONARY FUNCTION ($N^* = 44$)

	Δ FEV$_1$ (1)**%	Δ FEF$_{25\%-75\%}$VC (1/sec)
NO$_2$	0.26	0.33
	p = .08	p = .03
SO$_2$	0.13	-0.01
	p = .40	p = .96

*N = number of persons
**Δ = linear change

TABLE 7. CORRELATION COEFFICIENTS BETWEEN MEAN
POLLUTANT EXPOSURE AND MEAN CHANGE
IN PULMONARY FUNCTION

	Δ FEV$_1$ (1)**%	Δ FEF$_{25\%-75\%}$VC (1/sec)
Non-Asthmatics ($N^* = 14$)		
NO$_2$	0.61	0.37
	p = .02	p = .19
SO$_2$	0.78	0.29
	p = .001	p = .32
Asthmatics ($N^* = 30$)		
NO$_2$	0.19	0.33
	p = .31	p = .07
SO$_2$	-0.06	-0.19
	p = .77	p = .32

*N = number of persons
**Δ = linear change

TABLE 8. CORRELATION COEFFICIENTS BETWEEN MEAN POLLUTANT EXPOSURE AND MEAN LEVEL OF PULMONARY FUNCTION (N* = 44)

	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (1/sec)
NO_2	0.43	0.32
	p = .004	p = .03
SO_2	0.38	0.24
	p = .01	p = .11

*N = number of persons

TABLE 9. CORRELATION COEFFICIENTS BETWEEN MEAN POLLUTANT LEVEL AND MEAN LEVEL OF PULMONARY FUNCTION

	Non-Asthmatics (N*=14)		Asthmatics (N*=30)	
	FEV_1(1)	$FEF_{25\%-75\%VC}$ (1/sec)	FEV_1(1)	$FEF_{25\%-75\%VC}$ (1/sec)
NO_2	0.35	0.03	0.39	0.45
	p = .22	p = .92	p = .03	p = .01
SO_2	0.27	0.04	0.40	0.30
	p = .34	p = .89	p = .03	p = .11

*N = number of persons

FIGURE 1.   FREQUENCY DISTRIBUTIONS

PERSONAL

(N = 379)

MEAN = 22.6

S.D. = 12.1

NO$_2$ (ppb)

GAGE MOE

(N = 379)

MEAN = 32.2

S.D. = 16.1

NO$_2$ (ppb)
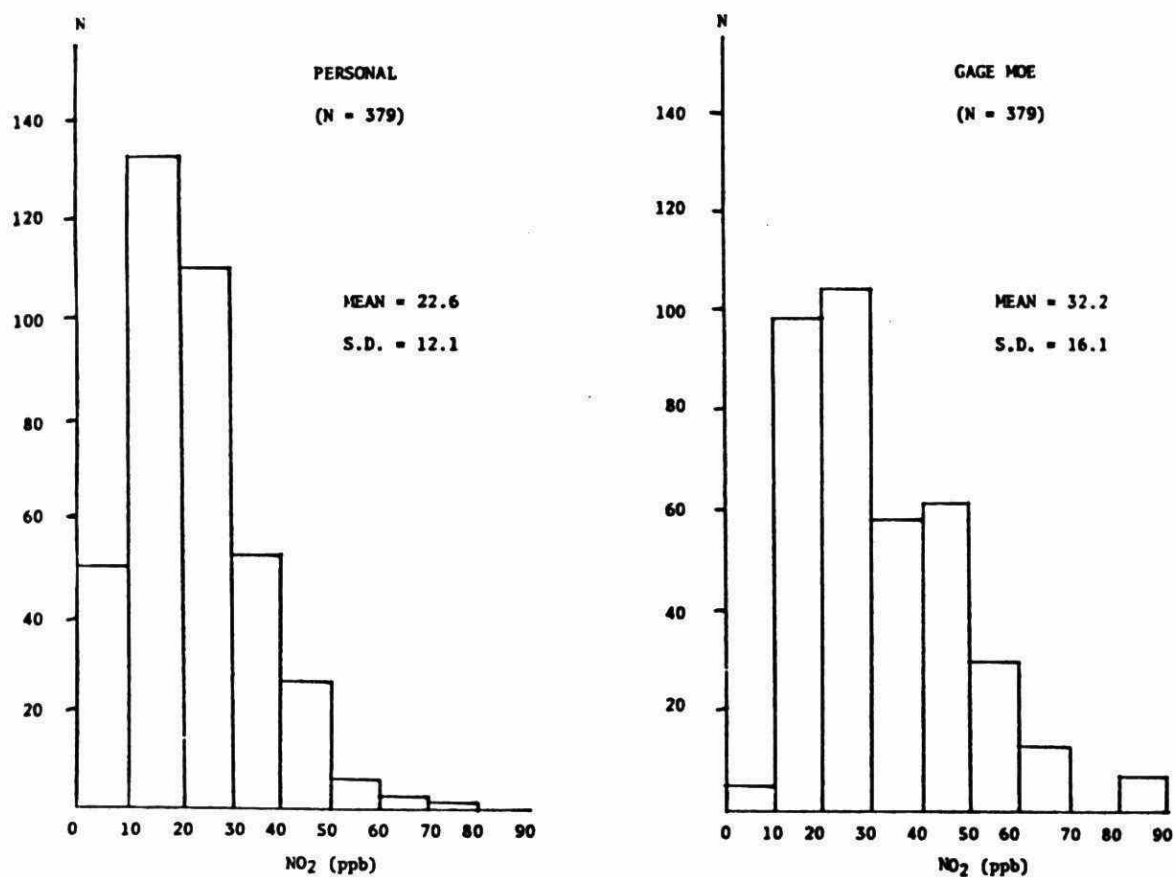
FIGURE 2.   FREQUENCY DISTRIBUTIONS

65

PERSONAL

(N = 379)

MEAN = 2.4

S.D. = 5.0

SO$_2$ (ppb)

GAGE MOE

(N = 379)

MEAN = 8.3

S.D. = 8.6

SO$_2$ (ppb)
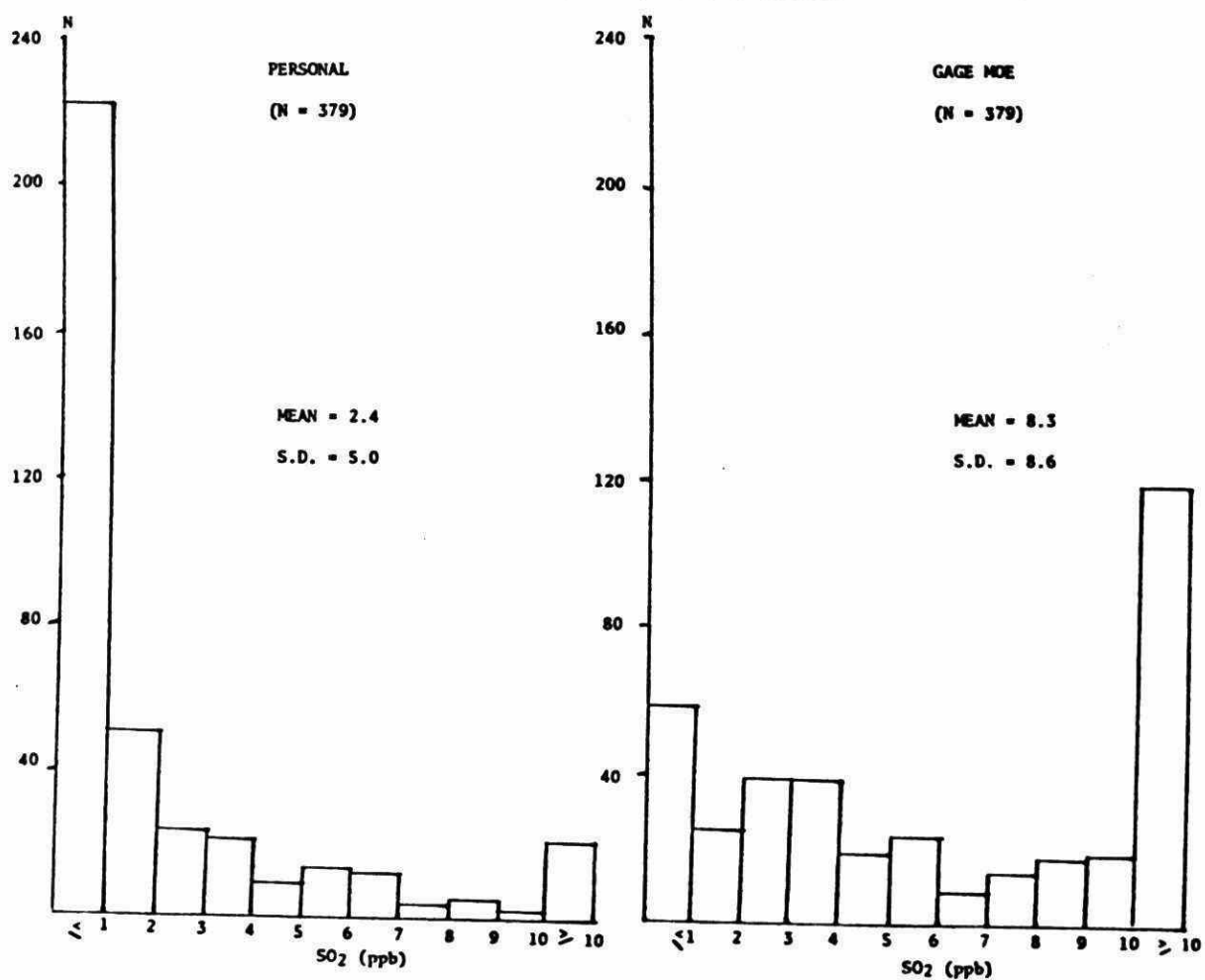
Change in FEF$_{25\%-75\%VC}$
(AM - PM) (l/sec)

FIGURE 3. RELATIONSHIP BETWEEN SO$_2$ CONCENTRATION AND DAILY CHANGE IN FEF$_{25\%-75\%VC}$ FOR ONE INDIVIDUAL

Slope = 0.42

r = 0.60

(p < .01)

N = 18

[SO$_2$] ppb
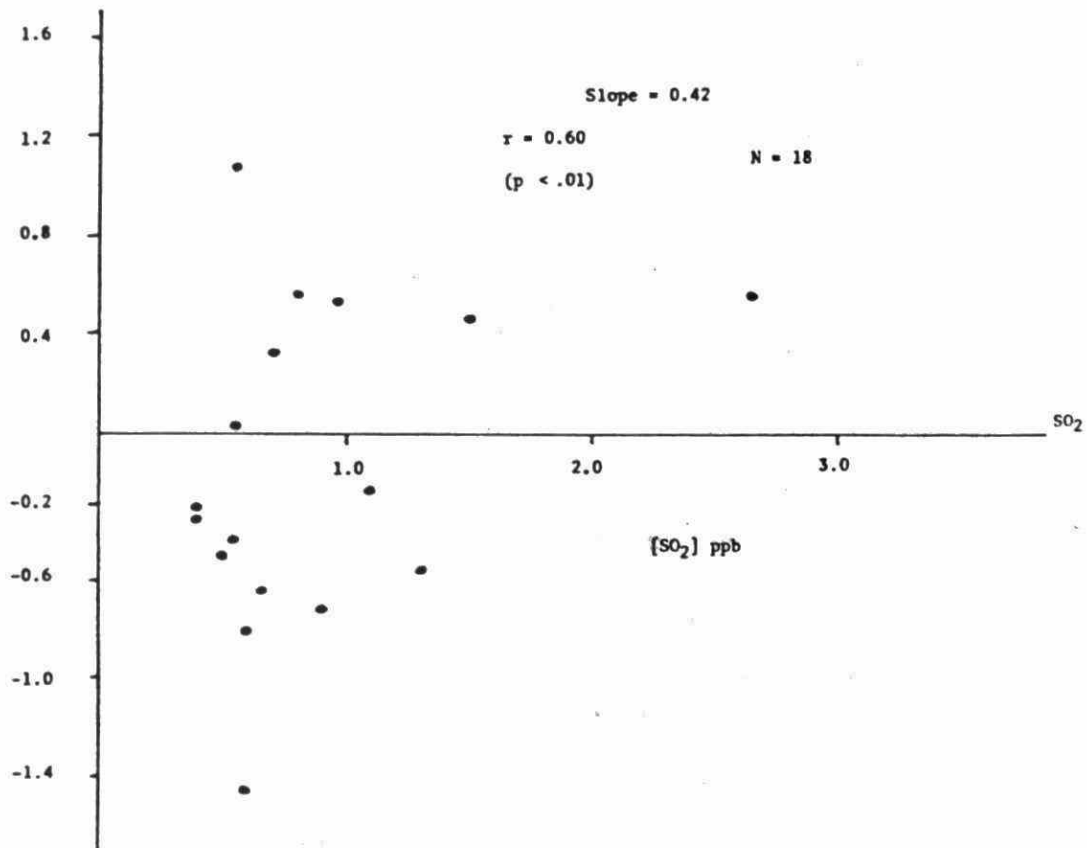
FIGURE 4. RELATIONSHIP BETWEEN THE LOG NO$_2$ CONCENTRATION AND THE DAILY FEF$_{25\%-75\%VC}$ FOR ONE INDIVIDUAL

Daily mean
FEF$_{25\%-75\%VC}$ (l/sec)

log NO$_2$
(log ppb)

Intercept = 4.858867

Slope = -.63832

R = -.77
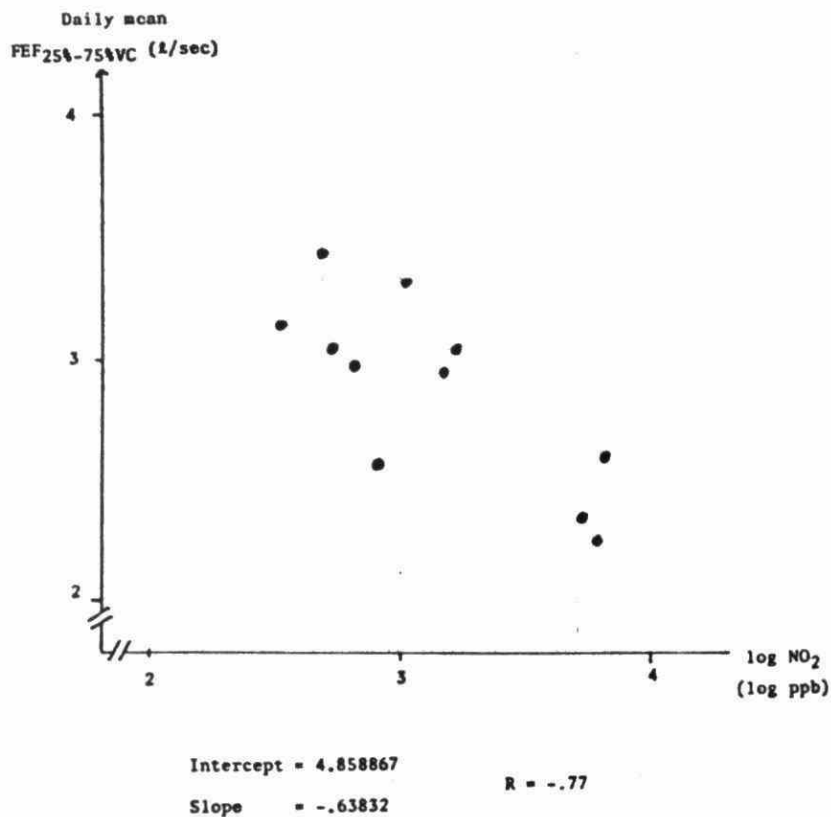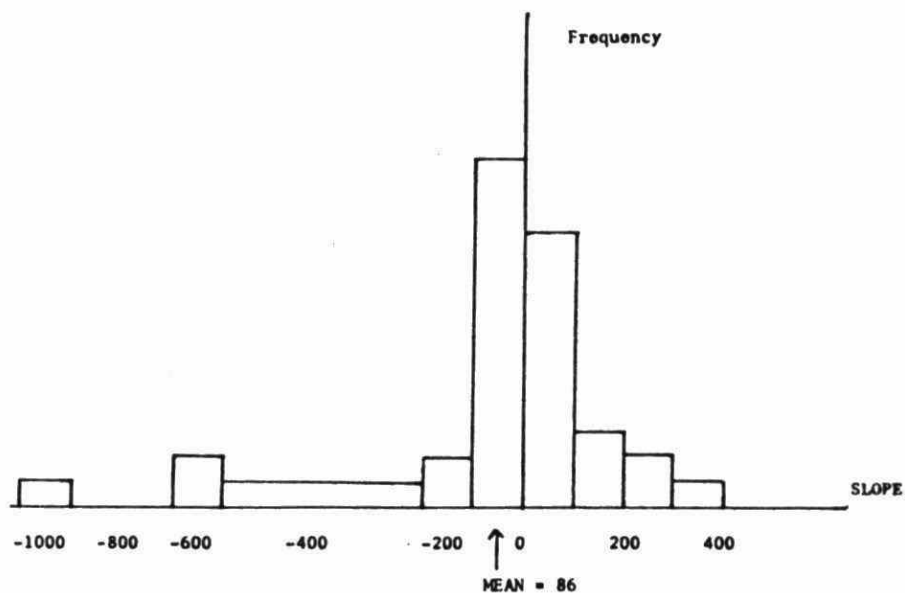
FIGURE 5.

DISTRIBUTION OF INDIVIDUAL SLOPES RELATING DAILY $FEF_{25\%-75\%VC}$
AND DAILY LOG NO_2 MEASURED BY THE GAGE MONITOR AT THE MINISTRY
SITE (N = 39).

MEAN SLOPE		-86
STANDARD DEVIATION		0.26
t = -2.1	p = 0.05	

82

FIGURE 6.
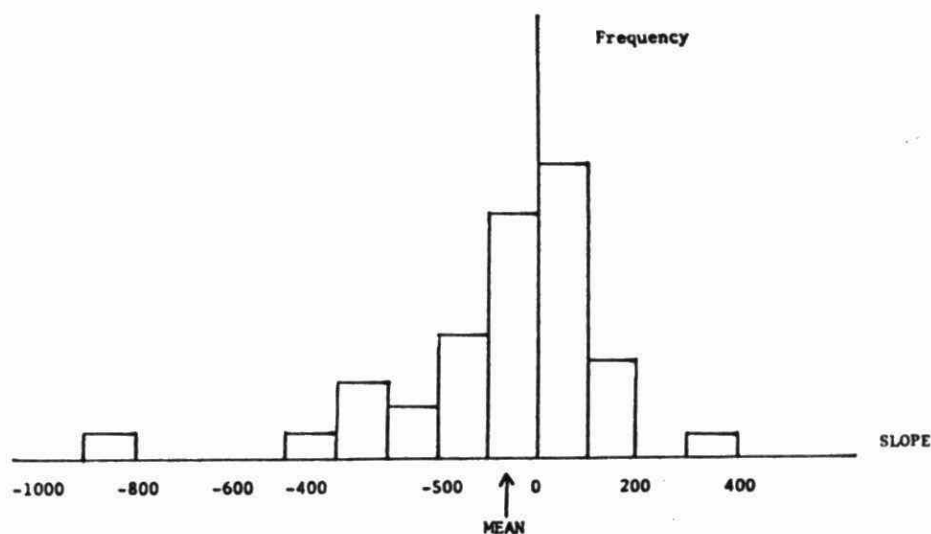
DISTRIBUTION OF INDIVIDUAL SLOPES RELATING DAILY $FEF_{25\%-75\%VC}$ AND
DAILY LOG SO_2 MEASURED BY THE GAGE MONITOR AT THE MINISTRY SITE
(N = 39).

MEAN SLOPE		-71
STANDARD DEVIATION		0.20
t = -2.16	p = 0.04	

A MASS SPECTROMETRIC STUDY OF SELECTED

AIR POLLUTANTS

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ABSTRACT:

Among the growing list of environmental pollutants present in the atmosphere
is a class of multiple fused-ring aromatics known as Polycyclic Aromatic
Hydrocarbons (PAH). Only a small number of these PAHs and related heterocycles
such as anthracene,pyrene and carbazole are produced in pure form for industrial
purposes, however many are produced whenever organic material is heated above
700 ° C, such as in pyrolysis or incomplete combustion. Furthermore, if the
starting materials contain heteroatoms such as nitrogen,oxygen and sulphur the
the products will contain a variety of related heteroaromatic compounds.

In general, PAHs are found everywhere in the environment and concern
over their presence stems from the carcinogenicity of some members of this
group such as benzo[a]pyrene. More recently concern has grown due to a degree
of excess carcinogenicity from both urban air and exhaust particulates from
combustion engines, which could not be accounted for solely on the basis of
their measured content of benzo[a]pyrene and other related PAH. It has now
been shown that PAHs can combine with other priority pollutants such as nitrogen
oxides(NO_x), sulphur oxides(SO_x), ozone(O_3) and peroxy acetylnitrate(PAN).
Furthermore this process may be facilitated by the adsorption of PAHs onto the
surface of small particulate organic matter(POM) in the respirable(1 micron)
diameter range. Thus the problems in urban atmospheres can be compounded by
the production, from inactive PAH, of substituted PAHs which are active in the
Ames microsomic mutagenicity test.

It has been found that the reaction of benzo[a]pyrene with NO_2 at 300 ° C
gives rise to a variety of products including the mono-,di- and trinitro-
derivatives which are presumably mutagenic in nature, as well as quinone derivatives
some of which may be active and some of which are not. Prolonged heating of
benzo[a]pyrene(12 hr.) with a molar excess of NO_2 in the ratio 5:1 or greater
appears to result in the production of a lactone-quinone of molecular weight
300 amu. whose activity is unknown.

The storage of positively-charged benzo[a]pyrene and chrysene ions in
the presence of NO and NO_2 has shown them to be unreactive under the conditions
employed.

Introduction

A great deal of attention has been given over the past few decades to the major pollutants present in urban atmospheres. These include sulphur oxides, carbon monoxide, nitrogen oxides, photochemical oxidants and particulate matter. Recently however, interest has developed in better understanding the presence, transport and chemical properties of a group of so-called "non-criteria pollutants" which, although present in urban atmospheres at lower concentrations than the "criteria pollutants", are known to cause disproportionately severe health effects in experimental animals and possibly in man[1]. One example of a class of such pollutants is the Polycyclic Aromatic Hydrocarbons(PAH) which are produced whenever there is pyrolysis or incomplete combustion of organic matter. PAHs consist of three or more fused benzene rings in linear, angular or cluster arrangements as shown in Figure 1, and contain only carbon and hydrogen. Although only 8 are shown here there are as many as 200 different PAHs in the air, soil and water[2].

PAH formation proceeds by free radical mechanisms; radical species can combine rapidly at high temperatures or under pyrolytic conditions; reactive transients are stabilized by ring closure, condensation, dehydrogenation, Diels-Alder reactions, ring expansions and other pathways to yield complex polycyclic organic matter[3] such as benzo[a]pyrene depicted in Figure 2.

In Figure 3 is shown the reaction of benzo[a]pyrene in biological systems. The starting material, once into the body, is oxidized to a form known as the "ultimate carcinogen", an epoxy-diol which can then attach itself to a molecule of DNA with the resulting biological effect of cancer initiation.

There is an appreciable amount of information in the literature about the biological reactions of many PAHs as well as the analytical methods employed for their detection. Unfortunately there is not only a lack of reliable information on atmospheric transformations of these chemicals but some actual misinformation: in two papers, from 1971[4] and 1976[5] it is reported that PAHs as air pollutants are "chemically inert and are thus removed from the air only by rain or the slow sedimentation of the particulate." Although the evidence is limited it is clear that many polycyclics, such as benzo[a]pyrene, are highly reactive compounds. These undergo a variety of atmospheric reactions both thermal and photochemical, with a number of co-pollutants. Furthermore, there is some evidence that these reactions may be facilitated by adsorption onto particulate matter in the respirable(1 micron) diameter range. In a 1980
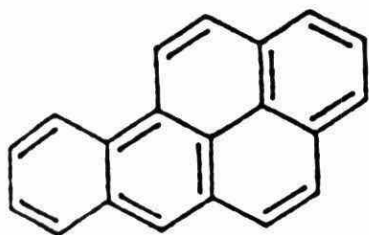
Benzo[a]pyrene
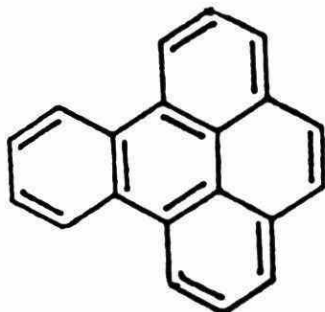
Benzo[e]pyrene
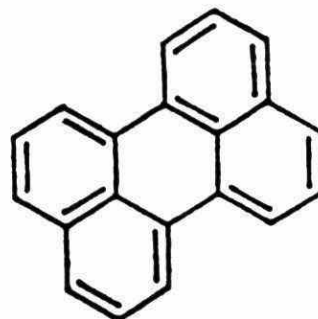
Perylene
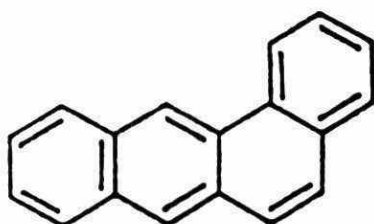
Benz[a]anthracene
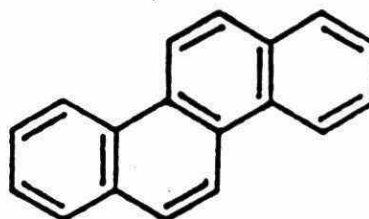
Chrysene
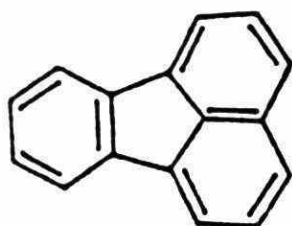
Fluoranthene
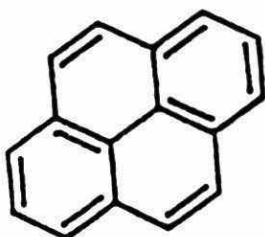
Pyrene
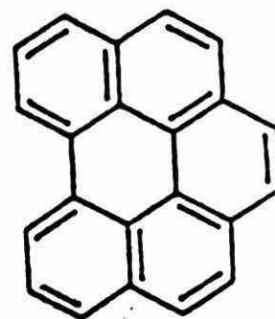
Benzo[ghi]perylene

Figure 1. Polycyclic Aromatic Hydrocarbons Present in Combustion-Related
Particulate Organic Matter.

Figure 2. Formation of benzo[a]pyrene
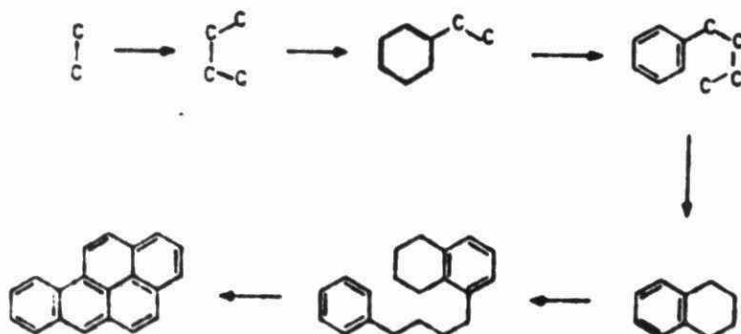
Figure 3. Biological oxidation of benzo[a]pyrene
MO is MonoOxygenase EH is EpoxyHydrolase
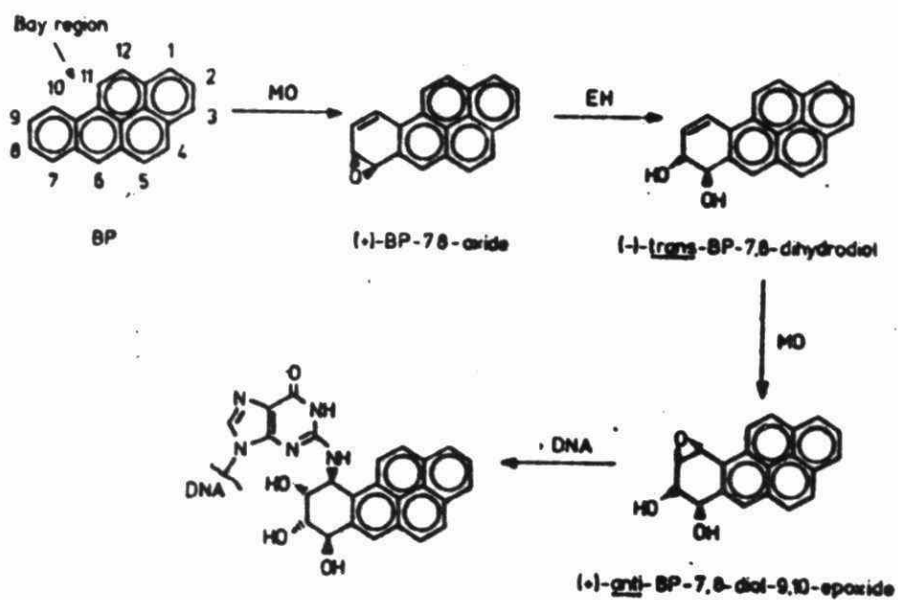
paper by Dr. J.N.Pitts et al, it is stated that" First, although organic extracts of polycyclic organic matter from ambient air and the exhaust from spark ignition engines are known to be carcinogenic, their activity can be significantly greater than would be expected on the basis of their known PAH content. Second, application of the Ames Salmonella mutagen assay to such extracts... has demonstrated a significant level of direct mutagenicity ... which is not ascribable to the promutagenic PAH."

Experimental Work and Results

Preliminary studies have been conducted of the two PAH benzo[a]pyrene and chrysene. The conventional electron impact mass spectra of these compounds obtained on an AEI MS12 magnetic sector mass spectrometer are quite similar. Both show a large peak corresponding to the molecular ion at 252 amu for benzo-pyrene and 228 amu for chrysene. These are accompanied by peaks at M-26 and M-52 indicating loss of one and two ethylene units, respectively, and the associated metastable peaks. The stability of these ions is evidenced by the fact that there also appear on the spectra peaks associated with doubly,triply and even quadruply-charged ions as well as doubly-charged metastable peaks.

Neither substance shows any change upon heating to 400°C in vacuo. In the presence of oxygen, both compounds react little at 100°C but are severely degraded upon heating to 400° for 12 hours.

Experiments were performed involving chrysene and oxygen below the ratio of 21:1 which would theoretically give only CO_2 and H_2O. All samples were heated to 400°, held for 4 hours and allowed to cool. All samples gave similar spectra regardless of the amount of oxygen present indicating that although reaction occurs in oxygen-rich conditions after 12 hours, there is little reaction in oxygen-lean conditions after 4 hours.

In the vapour condensed from the reaction tubes there were observed peaks corresponding to water, carbon dioxide and phenol.There were no traces of oxygen atoms or molecular oxygen being added to the parent molecule upon heating but rather a change in the fragmentation pattern which could indicate a skeletal rearrangement; peaks at m/z 215,216 and 217 which had been present in the mass spectrum of the starting material disappeared and a new peak at 218 appeared. There was also a decrease of m/z 242 and 266(impurities) and an increase in 246 and 280 possibly due to formation of higher-molecular-weight PAH.

In the case of benzo[a]pyrene when irradiated with ultraviolet light in the presence of oxygen, two hydrogen atoms are replaced by oxygen resulting

in the formation of benzo[a]pyrene quinone isomers.

Experiments were also performed in sealed tubes containing 2.5mg PAH and nitrogen dioxide in 1:1 and 1:10 ratios which were heated to 300°C and held overnight.The resulting mass spectra showed a variety of reaction products from both chrysene and benzo[a]pyrene and are reproduced in Figures 4 and 5.

In a 1:1 ratio with nitrogen dioxide chrysene produces a number of mass spectral peaks which include the mononitro derivative at m/z 273 and two related peaks at 243 and 215 which are either simple electron impact fragments or the stable products chrysene ketone and the ketone minus carbon monoxide. The base peak m/z 230 is presumably a rearrangement product of chrysene. The fragment-ation pattern in the spectrum at m/z 224 to 227 probably indicates the presence of a number of mononitro isomers.

In a 1:10 ratio with nitrogen dioxide the sample was severely degraded and the mass spectrum, shown in Figure 4(c), contained large peaks at m/z 50,76, 104,medium peaks at m/z 122,148 and 168, and all other peaks being less than 1% of the base peak.

The electron impact spectrum of benzo[a]pyrene is reproduced in Figure 5(a).The reaction with a 1:1 ratio with NO_2 gave rise to the spectrum 5(b). Re-action in a 1:10 ratio produced the spectrum 5(c) which, surprisingly, consisted of only 4 large peaks in excess of m/z 170 and a few minor fragment peaks of low intensity. A series of reactions were then carried out in which the ratio of NO_2 was reduced successively in order to investigate more closely the reactions taking place. The analyses of these products were used to create the profile of reaction products in Figure 6.

On the ordinate of Figure 6. is plotted the height of the mass spectral peak of interest divided by the sum of heights of the other peaks in the spectrum above m/z 170, thus giving the intensity of each peak as a percentage of the total ionization. The lower mass ions were common to all the spectra and were omitted to simplify the calculations. These percentages are plotted against the molar ratio of nitrogen to benzo[a]pyrene in order to portray the products as a function of the amount of NO_2 present.

The above results were obtained after heating for 12 hours;benzo[a]pyrene however begins to react quickly with nitrogen dioxideand visibly darkens at room temperature in a few minutes. At 300°C this PAH is a colorless vapour but in the presence of NO_2 rapidly becomes a black liquid. Mass spectra of products formed just an hour after the start of the reaction(at 300°) indicate the same peaks present as in the 12-hour reaction but with the additional presence of the tri-

Figure 4(a).

CHRYSENE
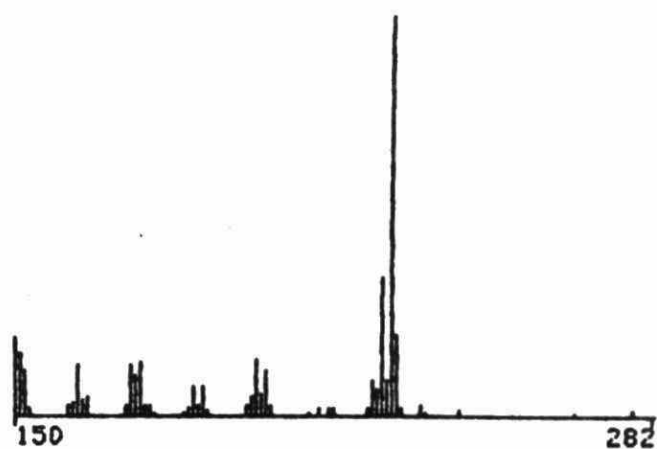
Figure 4(b).

CHRYSENE:NO_2

1:1
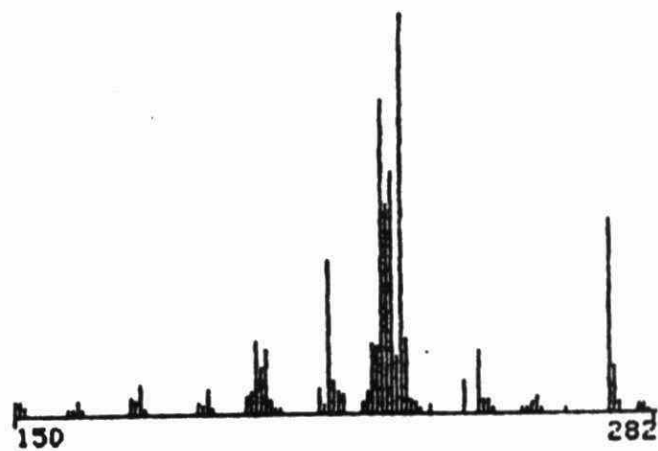
Figure 4(c).

CHRYSENE:NO_2

1:10
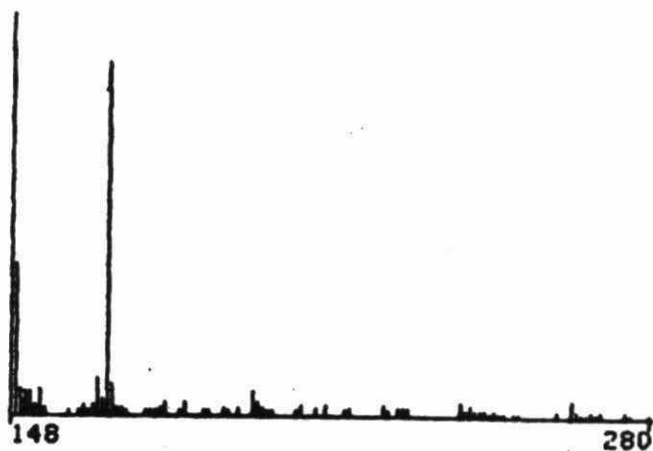
Figure 5(a).

BENZO[a]PYRENE

168   300
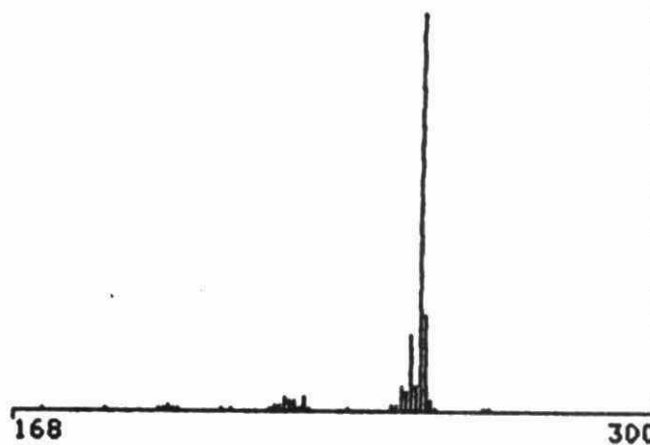
Figure 5(b).

BENZO[a]PYRENE:NO_2

1:1

170   302
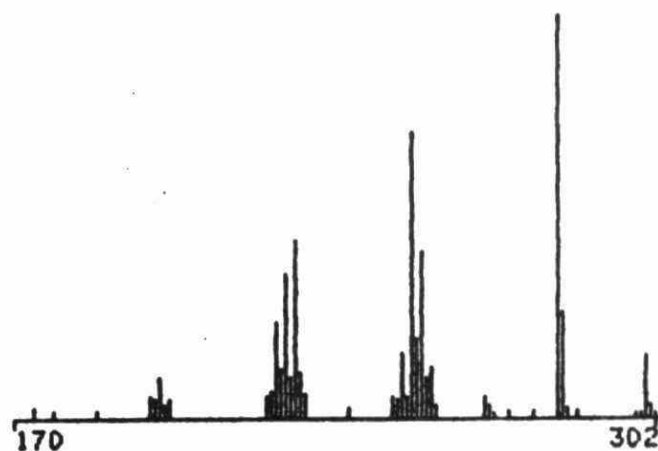
Figure 5(c).

BENZO[a]PYRENE:NO_2

1:10

170   302
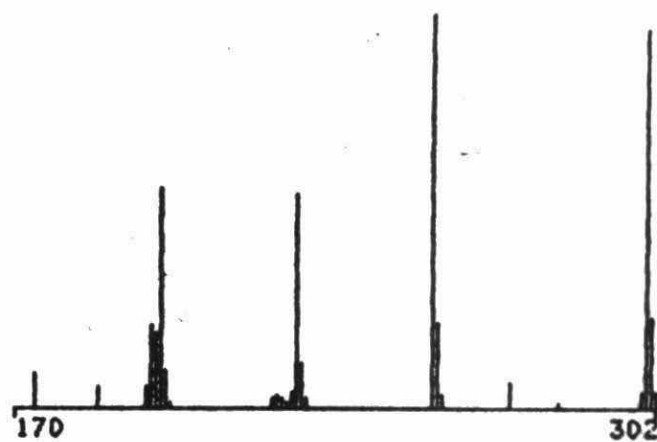
Figure 6.

Individual Ion Intensity(as % of total)

vs.

Molar Ratio of NO_2 to Benzo[a]pyrene

$NO_2/B[a]P$
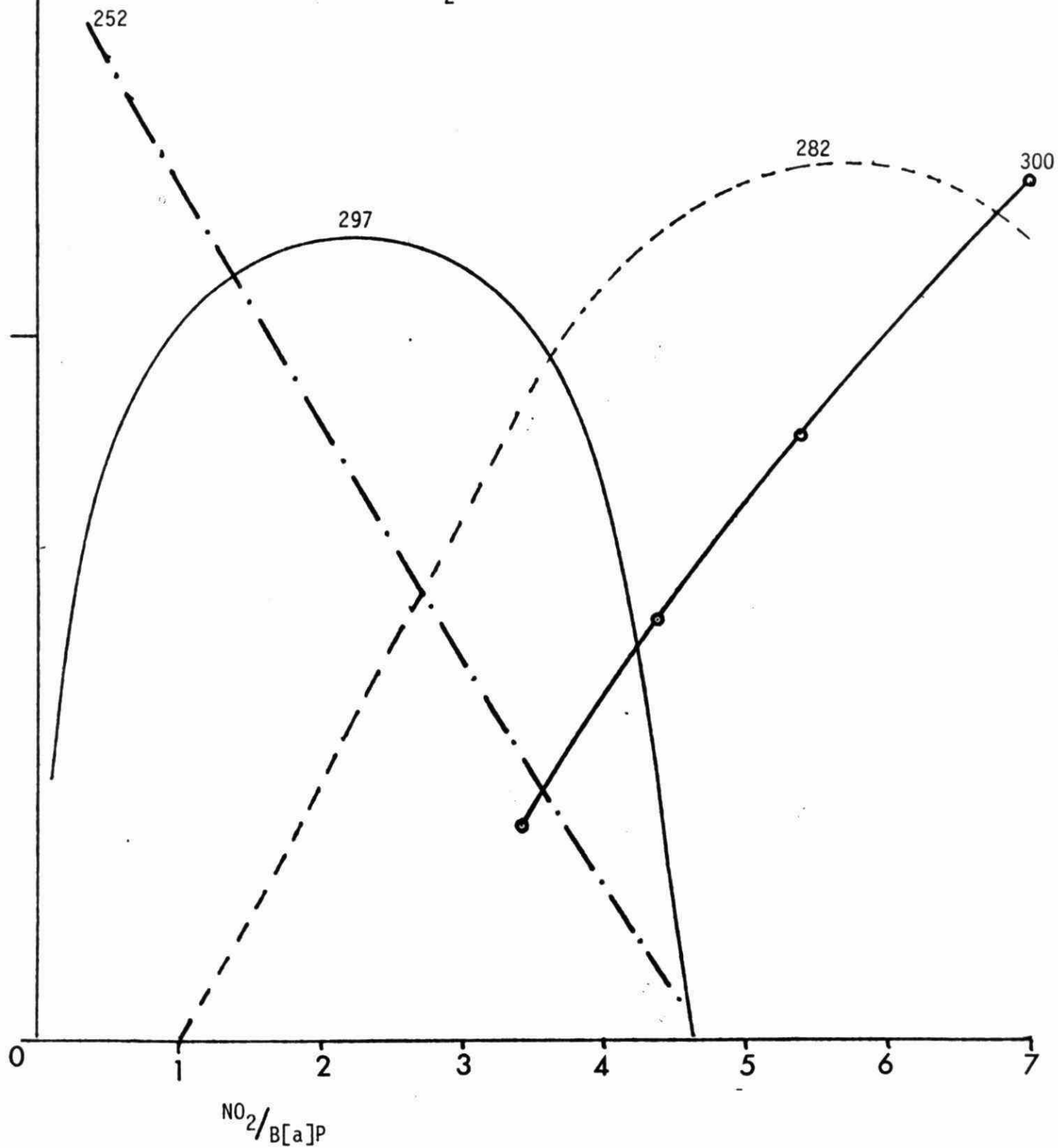
nitro and dinitro derivatives in addition to the mononitro product. The proposed reaction scheme which accounts for the major ion peaks in the spectra is reproduced in Figure 7. The ion peaks at m/z values of 252,297,342 and 282 represent the stable molecules benzo[a]pyrene,mononitro- and dinitro- benzo[a]pyrene and benzo[a]pyrene quinone dimers, respectively. At ambient temperature the major amount of nitration occurs at the 6- position[7] with minor amounts at the 1- and 3- positions. The position of the second nitro group in the dinitro derivative has not been established so it is not shown, in fig.7, attached at any position on the ring. The structures with m/z 267,312,239,284a and 254a may represent stable products or simply electron impact fragments.

The major ion resulting from the overnight reaction of benzo[a]pyrene and NO_2 where the NO_2 ratio is greater than 5 times that of the PAH has a mass of 300 amu and gives rise to peaks at 256,228 and 200 along with metastables for the accompanying losses. This product may be produced through the pathway depicted in Figure 8. Production of the lactone-quinone of mass 300 from the epoxide of mass 300 is similar to a thermolysis process reported in the literature[9].

Not shown in any of the figures but observed in some of the spectra were peaks corresponding to ions of masses between 480 and 520 which were possibly the dimer products of benzo[a]pyrene and NO_2.

Ion Chemistry of Benzo[a]pyrene and Chrysene

An attempt was made to study the ion/molecule reactions of benzo[a]- pyrene and chrysene in the quadrupole ion storage device shown schematically in Figure 9. The ion store, or QUISTOR, is an ion trap in which the inner surfaces of the top end cap,bottom end cap and ring electrodes are machined to produce a cavity which is bounded by hyperboloids of revolution about the z-axis;application of an RF voltage at 1.25 MHz to the ring electrode causes ions in the centre of the trap to undergo Lissajous-type trajectories and remain within the cavity.

The top end cap has a central hole to permit entrance of electrons from a heated tungsten filament which is mounted above the QUISTOR. The emission of the 70eV electrons is regulated in a pulsed fashion by means of a gate electrode between the filament and top end cap; the bottom end cap has a central hole in it through which ions exit into the source of a quadrupole mass filter. Positive ions are extracted by the periodic application of a 50μs negative pulse to the end cap; the time between the initial pulse of ionizing electrons and the ion extraction pulse is the storage time. The ring electrode has a 3mm. hole in it
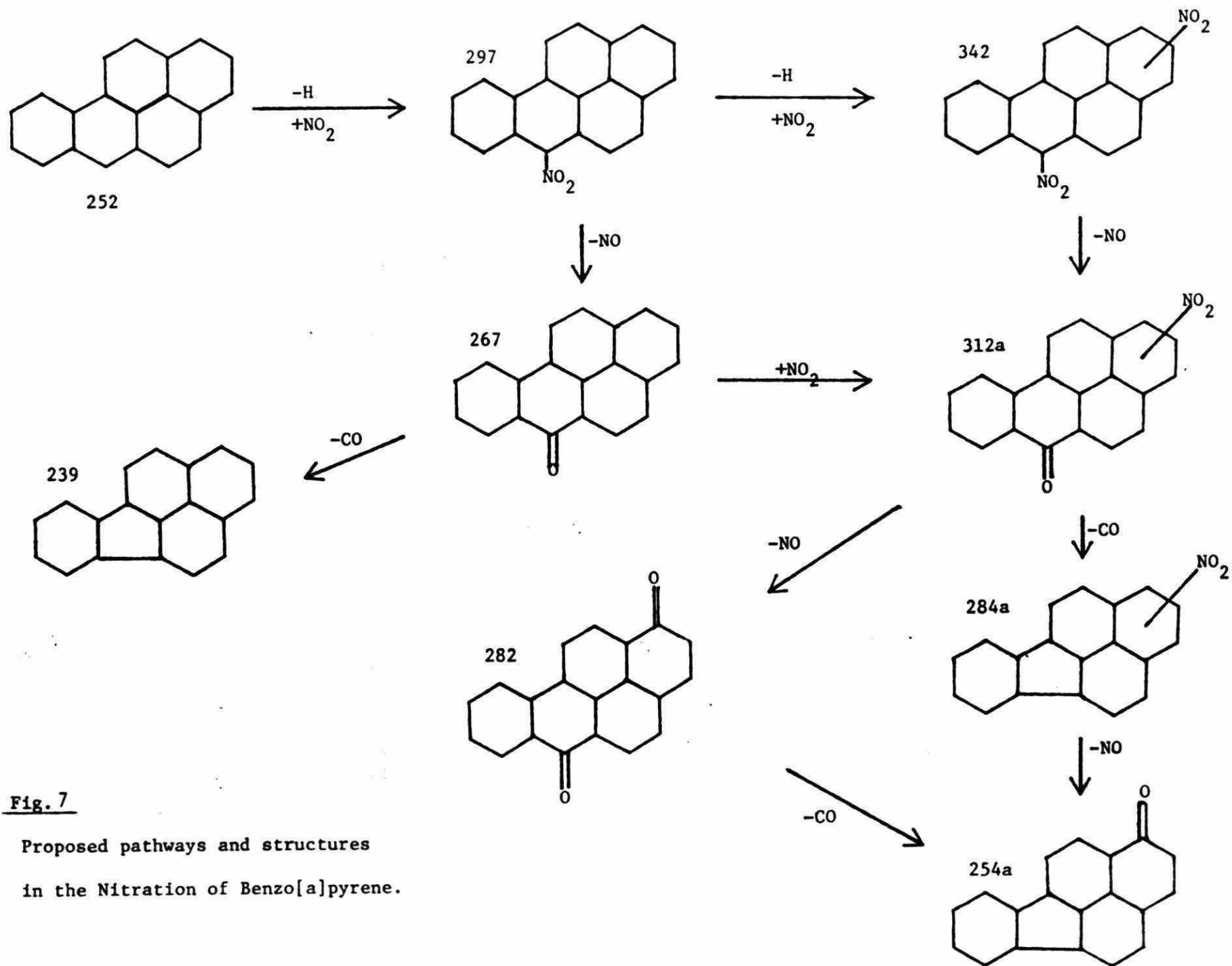
Fig. 7

Proposed pathways and structures

in the Nitration of Benzo[a]pyrene.

Fig. 8

Production of m/z 300 from Benzo[a]pyrene and NO₂
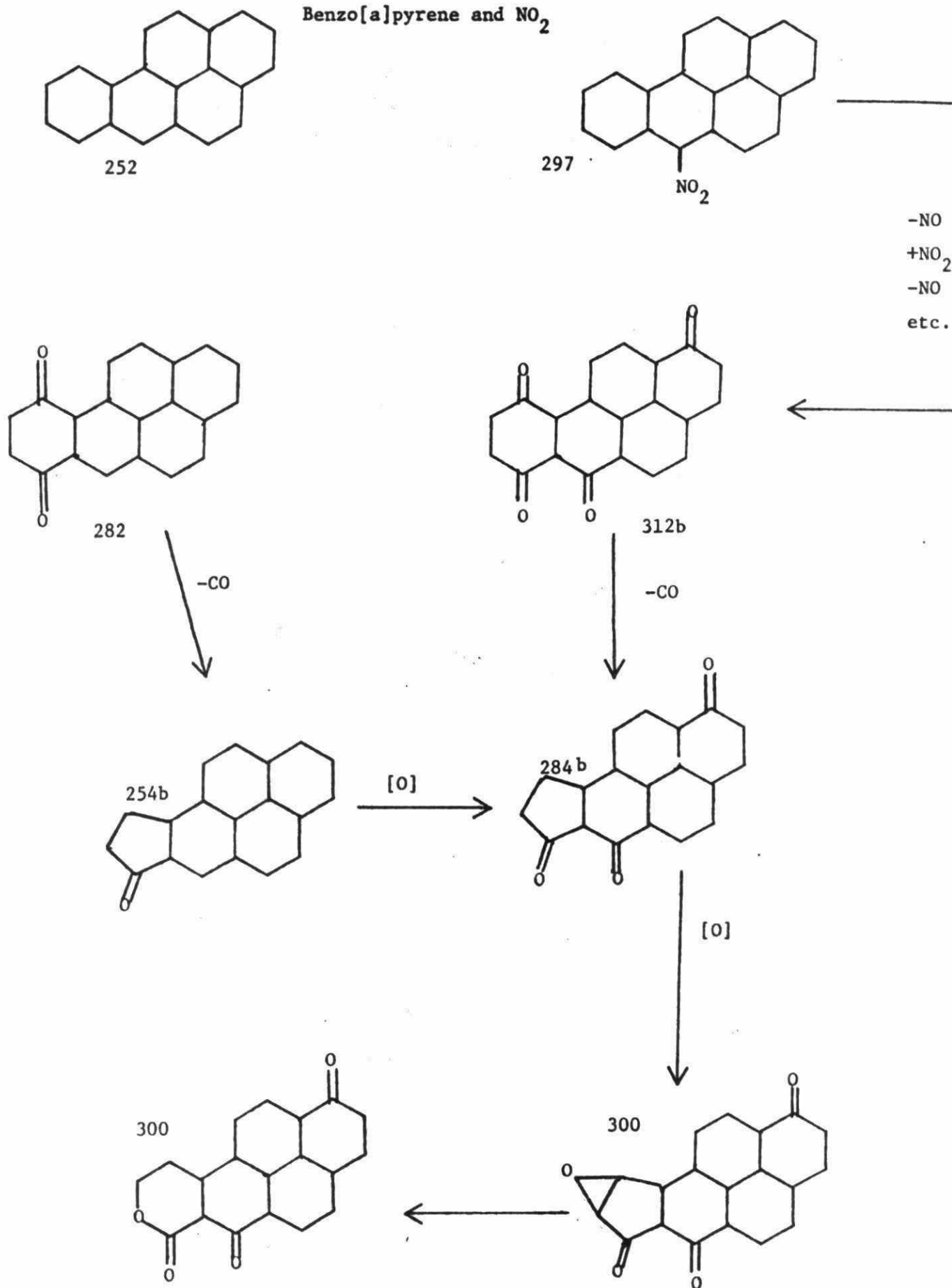
Figure 9. Quadrupole Ion Store QUISTOR

ELECTRON ENTRY

Z - AXIS

SAMPLE PROBE ENTRY

ION EXIT
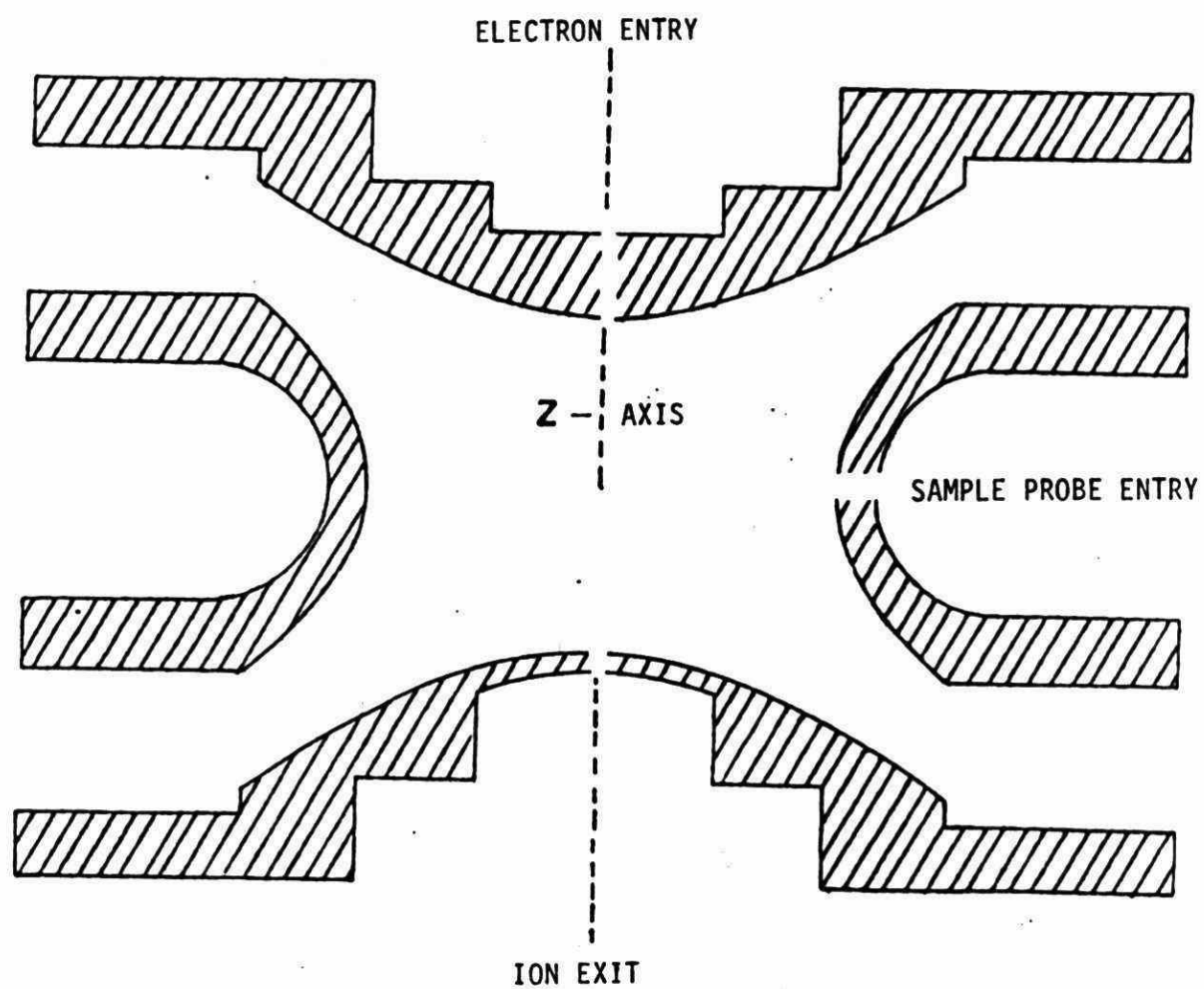
which is aligned with an external viewport in the vacuum tank. Two O-rings mounted at either end of this port form a seal against a 4.76mm. brass rod which can slide in and out of the tank while maintaining a pressure of about 0.02 mPa; affixed to the end of the brass rod is a Pyrex capillary tube fused just above one end to form a shallow depression. When a solid is placed in the depression it can be slid into the QUISTOR through the ring electrode where the ambient temperature vapourizes enough sample to obtain a mass spectrum employing either the electron impact source of the quadrupole mass filter or the QUISTOR in its storage mode.

Storage of ions produced by electron impact of the pure compounds benzo[a]pyrene and chrysene for periods up to 120 ms at pressures up to .5 mPa show very little reaction takes place in these systems. The parent ion accounts for more than 90% of the total ion signal. The smaller peaks are extracted from the background by repeated acquisition using a CYBORG ISAAC 91A digital interface and signal averaging employing an APPLE //e microcomputer.

Each PAH was subsequently stored in the QUISTOR at about 0.5 mPa and nitrogen dioxide or nitric oxide was admitted to a pressure of 10 mPa. The only change in the spectra observed was an increase in a peak at m/z 254 when NO was admitted with chrysene. This peak represented only about 0.25% of the total ionization and was only observed after signal averaging 100 sweeps of the quadrupole at high sensitivity. None of the results observed in these systems indicated a significant amount of reactivity of PAH ions under these conditions.

The lack of reactivity among these PAH ions is understandable on the basis of their aromatic character. The number of rings present in the system delocalize the electronic charge on the ion giving rise to enhanced stability. This stabilization effect is evident in the conventional electron impact spectra which displays quadruply-charged ions and multiply-charged metastables.

It is safe to say that some Polycyclic Aromatic Hydrocarbons, specifically benzo[a]pyrene and chrysene, are not inert in the presence of co-pollutants such as nitrogen dioxide, or oxygen and ultraviolet light in concert. Although their ions are not reactive under the conditions employed herein, it appears that these two, and probably many more polycyclics are highly reactive in the vapour phase and produce a large number of gaseous products, some of which will undoubtably prove to be harmful.

- <u>REFERENCES</u> -

1. Pitts,J.N.Jr.,"Photochemical and biological implications of the atmospheric reactions of amines and benzo[a]pyrene",Phil.Trans.R.Soc.Lond.A.290,551-576(1979)

2. Zander,M.,"Polycyclic Aromatic and Heteroaromatic Hydrocarbons",The Handbook of Environmental Chemistry, ed. O.Hutzinger,Vol.3,Part A.Anthropogenic Compounds 1980,Springer-Verlag,Berlin,Heidelberg,New York

3. Zedeck,M,Journal of Environmental Pathology and Toxicology 3:537-567,1980 Pathotox Publications

4. Quoted in Ref. 1

5. Quoted in Ref.1

6. Pitts,J.N.Jr. et al,SCIENCE,19 Dec. 1980,Vol. 210,p.1347

7. Pitts,J.N.Jr.,"Formation and Fate of Gaseous and Particulate Mutagens and Carcinogens in Real and Simulated Atmospheres",presented at the Karolinska Institute Symposium,Stockholm,Sweden,Feb. 1982,Environmental Health Perspectives

8. Pitts,J.N.Jr. et al,"Atmospheric Reactions of Polycyclic Aromatic Hydrocarbons: Facile Formation of Mutagenic Nitro Derivatives",SCIENCE,Vol.202,pp.515-519,1978

9. Lewars,E.G. and Young,A.B.,Tetrahedron Letters,4799(1979)

DEVELOPMENT OF A METHOD FOR MEASURING HYDROGEN PEROXIDE USING A TUNABLE DIODE LASER ABSORPTION SPECTROMETER

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ABSTRACT

Hydrogen peroxide is believed to be the most important oxidant in transferring SO_2 to H_2SO_4. It is also closely coupled to the highly reactive HO and HO_2 radicals which are central to all chemical processes in the atmosphere. Measurements of the gaseous concentration of this species are therefore important in furthering our understanding, not only of acid deposition in particular, but of atmospheric chemistry in general.

We are developing methods to apply our tunable diode laser absorption spectrometer to measure hydrogen peroxide. The spectrometer combines the high resolution of a tunable diode with the sensitivity provided by a long-path White cell. It should be highly specific to hydrogen peroxide without interferences from other gases.

Laboratory measurements with this system have shown detection limits of 1 to 2 ppbv. Although such detection limits would be adequate for polluted air improvements would be desirable for clean air measurements. We are developing computer data acquisition and analysis techniques to lower the detection limits. With the current version of this system the detection limit is better than 1 ppbv.

Field measurements also require suitable calibration and sampling techniques. We have developed a permeation device capable of delivering a flow rate of peroxide in the desired range which remains constant for periods of a week or more. A photometric analytical procedure has been perfected for determining the concentration of the calibration gas.

Tests have revealed that metals and epoxy glues remove peroxide from the gas stream while translucent Teflon, glass and the calcium fluoride windows do not. The sampling system is being constructed of these inert materials.

Hydrogen peroxide is believed to be the most important oxidant which converts S(IV) to S(VI) in the atmosphere. The processes are believed to be :

$$SO_2(g) + H_2O = SO_2.H_2O(aq) \qquad (1)$$

$$SO_2.H_2O(aq) = H^+ + HSO_3^- \qquad (2)$$

$$HSO_3^- + H_2O_2(aq) \rightarrow SO_4^{2-} + H^+ + H_2O \qquad (3).$$
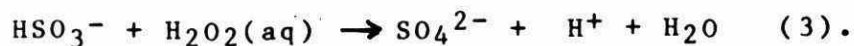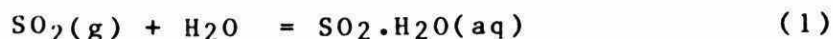
H_2O_2 is believed to be formed primarily from the recombination of HO_2 radicals:

$$HO_2 + HO_2 \rightarrow H_2O_2 \qquad (4)$$

During the daytime H_2O_2 also produces HO radicals by photolysis:

$$H_2O_2 + h\nu(<340 \text{ nm}) \rightarrow 2 \text{ HO}$$
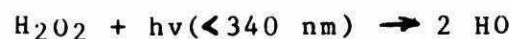
It therefore acts to convert HO_2 radicals to HO radicals. The HO radical plays a central role in the chemistry of photoxidants and acid deposition.

For these reasons, measurement of H_2O_2 in the atmosphere has received top priority. To date no measurement technique for this species has been demonstrated.

Infrared absorption presents an attractive opportunity for such measurements. Hydrogen peroxide has a well developed infrared absorption spectrum in the 8 u region. Very high resolution is required, however, to prevent interferences from other atmospheric constituents, particularly water vapour, N_2O and CO_2 which also absorb in this spectral region. The necessary resolution is available with the tunable diode laser absorption spectrometer (TDLAS) which we have developed.

The TDLAS has a number of advantages for atmospheric measurements. It is a passive technique with a response time of less than 1 minute, permitting in situ, real time measurments.

Because of its high resolution it provides positive identification of the target species with no interferences from other atmospheric species. By utilizing the long path length of a White cell it is able to detect most molecules in the fractional ppbv level.

A mobile TDLAS system has been constructed and used for field measurements of NO, NO_2 and HNO_3 (Schiff et al 1983). A schematic of this system is shown in Fig.1. The air is sampled through an all teflon inlet line into the White cell. A restriction at the inlet and a servo valve at the outlet maintain a constant air flow through the White cell. They also maintain the pressure in the cell at 25 Torr which reduces the pressure broadening of the line to an extent that makes interferences by other gases highly unlikely.

Calibration gases are added at known concentrations at the sampling inlet. Any losses that may occur in the sample line or in the White cell are compensated by this procedure.

The frequency at which a diode will lase depends on its temperature and the current passing through it. These diode typically operate somewhere in the 20 to 60 K range. A Helium cryocooler and a small heater maintain the appropriate temperature. The frequency of the diode can be scanned over a range of about 0.5 wavenumbers by changing the current passing through it. The laser beam is focussed into the White cell where it traverses a path length of 24 m. It is then focussed onto a HgCdTe detector. For initial set-up and spectroscopy a 10 cm cell containing the target gas at high concentration can be placed in the beam. The He-Ne laser is used initially to align the optics.

The spectrometer can be operated either in an amplitude modulated (AM) or a frequency modulated (FM) mode. In the AM mode

the beam is chopped mechanically and the transmitted power detected at the chopping frequency. However, since the observed absorption features represent small differences between large power signals this method is limited to absorptions of about 1%.

To overcome this difficulty the spectrometer is operated in the FM mode. A 2 kHz sine wave is applied to the laser current, producing a frequency-modulated laser output. Detection is made at twice this frequency. A comparison of the the signals obtained is exemplified in Fig.2. Since only changes in laser power are detected a great deal of the noise is eliminated. Absorptions as low as 10^{-5} are detectable which, for a 24 m path length correspond to a detection limit in the fractional ppbv range. However, since information about the initial power is lost calibration is required to relate the signal to concentration.

The application of this technique to the measurement of a particular atmospheric species has three components: (1) The selection of the best rotational-vibrational line. The choice is usually a compromise between line strength and operating characteristics of the diode. (2) Development of a suitable calibration procedure. (3) Development of a suitable sampling procedure.

Selection of Spectral Line

Three laser diodes were tested from which one was selected. It operated in a single mode that could be scanned across two relatively strong H_2O_2 absorption lines at 1264.59 and 1264.62 cm^{-1}. The integrated line strengths of these features are 0.4 and 1.8×10^{-20} cm^{-1} respectively. The stronger line has 60% of the linestrength of the strongest line in the H_2O_2 spectrum which

was unavailable with this diode. By comparison with our work on the
nitrogen oxides this linestrength implies a detection limit for
H_2O_2 of about 1.5 ppbv.

Figure 3 shows the FM signal for air containing 7 ppbv of
H_2O_2 at the 2 selected wavelengths. Scans across these lines,
with and without added peroxide are shown along with the limiting
beam noise when the laser is held at a fixed wavelength. The
detection limit is better than 2 ppbv as anticipated.

Calibration Procedure

Our philosophy of calibrating the instrument by adding a known
concentration of the target gas at the air inlet requires a stable
source of that gas in the ppmv range. Mixtures in this concentration
range in steel or aluminum cylinders can be used for stable,
non-polar gases such as NO, but are not suitable for H_2O_2.

We have successfully used permeation devices for the polar
gases NO_2 and HNO_3. These devices are based on permeation from a
high concentration source through a solid interface into a carrier
gas stream. If the temperature of the device is carefully controlled
the permeation rate remains constant for long periods of time.
Liquids are generally used to provide the high concentration within
the device and Teflon is the favoured material for the solid
interface. Because of the low vapour pressure of even a 90% H_2O_2
solution the permeation rate through Teflon was too low for our
purposes.

Higher permeation rates were achieved with low density
polyethylene tubing. To prevent decomposition of H_2O_2 by
impurities on the surface of the tubing it was cleaned with a hot
solution of 50% H_2O_2 and sulfuric acid. A 4 m coil of this

tubing, 0.32 mm O.D.,0.16 mm I.D. is immersed in a 90% H_2O_2 solution. At a temperature of 22 C and with a carrier gas flow of 20 standard cm^3 min^{-1} a concentration of 19.6 ppmv was obtained which remained stable for more than 1 week. This device therefore appears to be very suitable for our purposes.

To serve as a calibration source an analytical technique is required to provide accurate values for the permeation rate. The technique used is based on the colour change which occurs when H_2O_2 complexes with a solution of Ti(IV) (Pilz and Johann, 1974).

H_2O_2 is very soluble in water, having a Henry's Law constant of 10^5 molar atm^{-1} (Martin and Damschen, 1981). Because the complexing reaction with Ti(IV) removes aqueous H_2O_2 gaseous H_2O_2 can be effectively scavenged by this solution.

Interferences can occur when attempts are made to absorb H_2O_2 from real air. For example, the presence of SO_2 in the air can destroy H_2O_2 by the reaction sequence (1) - (3). By contrast, bubbling air containing O_3 through water has been shown to produce aqueous H_2O_2. The analytical technique is therefore not suitable for measuring H_2O_2 in real air but is suitable for analysing the gas from the permeation device which uses pure N_2 as the carrier gas.

Aqueous H_2O_2 reacts quantitatively with Ti(IV) to form a complex which is stable at pH $\leq$ 1. The nature of the complex is uncertain but the evidence is overwhelming that it contains a single peroxide molecule. Thus, in the presence of excess Ti(IV) quantitative conversion of H_2O_2 to the complex form can be assumed. The Ti(IV) solution is colourless, having an absorption in

the u.v. with a maximum below 330 nm. The peroxide complex is red-orange with a maximum absorption at 410-415 nm. The maximum absorption coefficent is 735 M^{-1} with no contribution from the Ti(IV) solution itself. Optical absorption at 410 nm can therefore be used to determine the peroxide complex and consequently the gaseous H_2O_2 concentration.

The analytical procedure used is as follows. 11.6 ml of pure $TiCl_4$ is dissolved in 50 ml of concentrated (37%, 12M) HCl. Care must be taken at this stage since $TiCl_4$ reacts vigorously with moisture to generate HCl and a great deal of heat. The resulting solution is bright yellow and keeps indefinitely.

This solution is diluted ten-fold with distilled water to produce a solution (B) which is colourless and cannot be stored for more than a few days since it slowly decomposes with precipitation of TiO_2.

One ml of solution B is diluted with 20 ml of 3.7% (1.2 M) HCl. This solution is placed in a microimpinger and the calibrating gas stream bubbled through it until yellow coloration is observed. The sample is removed, the impinger washed with 3.7% HCl to dilute the solution to 50 ml. The absorbance is measured at 410 nm using a Bausch and Lomb visible/uv spectrophotometer. From this measurement, the known absorption coefficient of the complex and the flow rate of the gas through the impinger the concentration of H_2O_2 is determined.

Sampling Procedure

Since H_2O_2 is reactive and decomposes readily, studies were made of various materials to test their suitability for use in the sampling system. These tests were performed by inserting the

materials between our H_2O_2 source and the Ti(IV) solution. Metals such as stainless steel were found to remove significant amounts of the peroxide from the gas stream and do not appear to be conditioned. All epoxy resins and Torr seal react to remove the peroxide from the gas stream completely. Glass, translucent Teflon and dry calcium fluoride (used for cell windows) do not remove the peroxide from the gas stream provided that they are preconditioned for a few minutes with the gas mixture. The only sealant we have found suitable for optical windows is Apiezon W hydrocarbon wax.

The calibration and sampling system is currently being built from these inert materials and tests will be conducted to determine the conditioning time of this system to changes in H_2O_2 concentrations. This will determine the response time of this instrument for this gas.

Minimum Detection Limit

The detection limit of better than 2 ppbv mentioned earlier is acceptable for measurements in polluted air where the H_2O_2 concentrations higher than 10 ppbv have been reported (Kok et al, 1978). Concentrations in rural air, however, are likely to be below this detection limit and improvements must be made for regional scale measurements.

The most promising method for improving the detection limit appears to be by data processing. Much of the noise observed in the analog detection method shown in Figure 3 is frequency dependent and should be amenable to data averaging procedures to remove the background spectrum. Considerable efforts have been expended in creating software for such data handling. Figure 4 shows some preliminary results where curve C is obtained by subtracting by

microcomputer the background curve B from the curve A obtained with

a gas mixture containing 3.9 ppbv of H_2O_2. The detection limit

has been greatly improved by this procedure to well below 1 ppbv.

Further improvements are likely from our continuing software

development.

References

Kok, G.L., K.R. Darnall, A.M. Winer, J.N. Pitts Jr. and B.W. Gay, (1978) Envir. Sci. and Technol., 12, 1077.

Martin, L.R. and D.E. Damschen. (1981) Internat. J. Environ. Anal. Chem., 3, 257.
Pilz, W. and Johann I., (1974) Intern. J. Environ. Anal. Chem., 3, 257

Schiff, H.I. D.R. Hastie, G.I. Mackay, T. Iguchi and B.A. Ridley, (1983), Envir. Sci. and Technol. 17, 352A.

Bottled
air

-Pump

Inlet

Key

MV	motorized valve	D	HgTeCd infrared detector	FM2	mass flow meter– total flow	⊚	4 00 ppm NO in N₂ spectral-seal cylinder
P	Baratron pressure	IP	ion pump	PT 1	NO₂ permeation tube	N	Teflon needle valve
W	White cell	R	rotameter flowmeters	PT 2	HNO₃ permeation tube	C	collimating optics
He–Ne	helium–neon alignment laser	x	capillary	V	three-way valve		
LSA	laser source assembly	FM1	mass flow controller–NO flow	▨	solenoid valve		

FIGURE I. MOBILE TDLAS SYSTEM
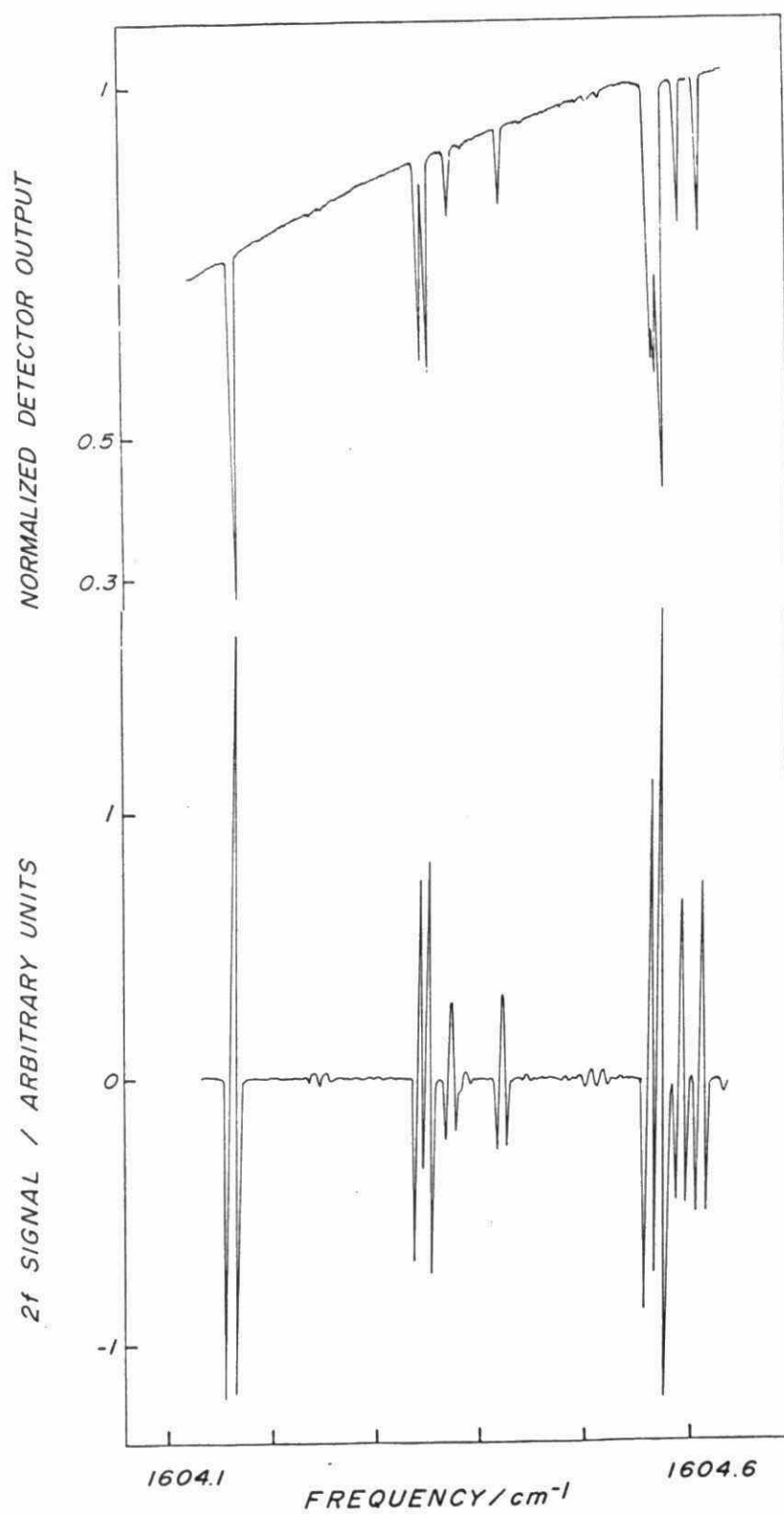
FIGURE 2. AM (TOP) AND FM (BOTTOM) ABSORPTION SPECTRA OF NO_2.

FIGURE 3. ANALOG DETECTION OF H_2O_2: THE DASHED VERTICAL LINES INDICATE THE MAXIMA OF THE H_2O_2 ABSORPTION FEATURES.
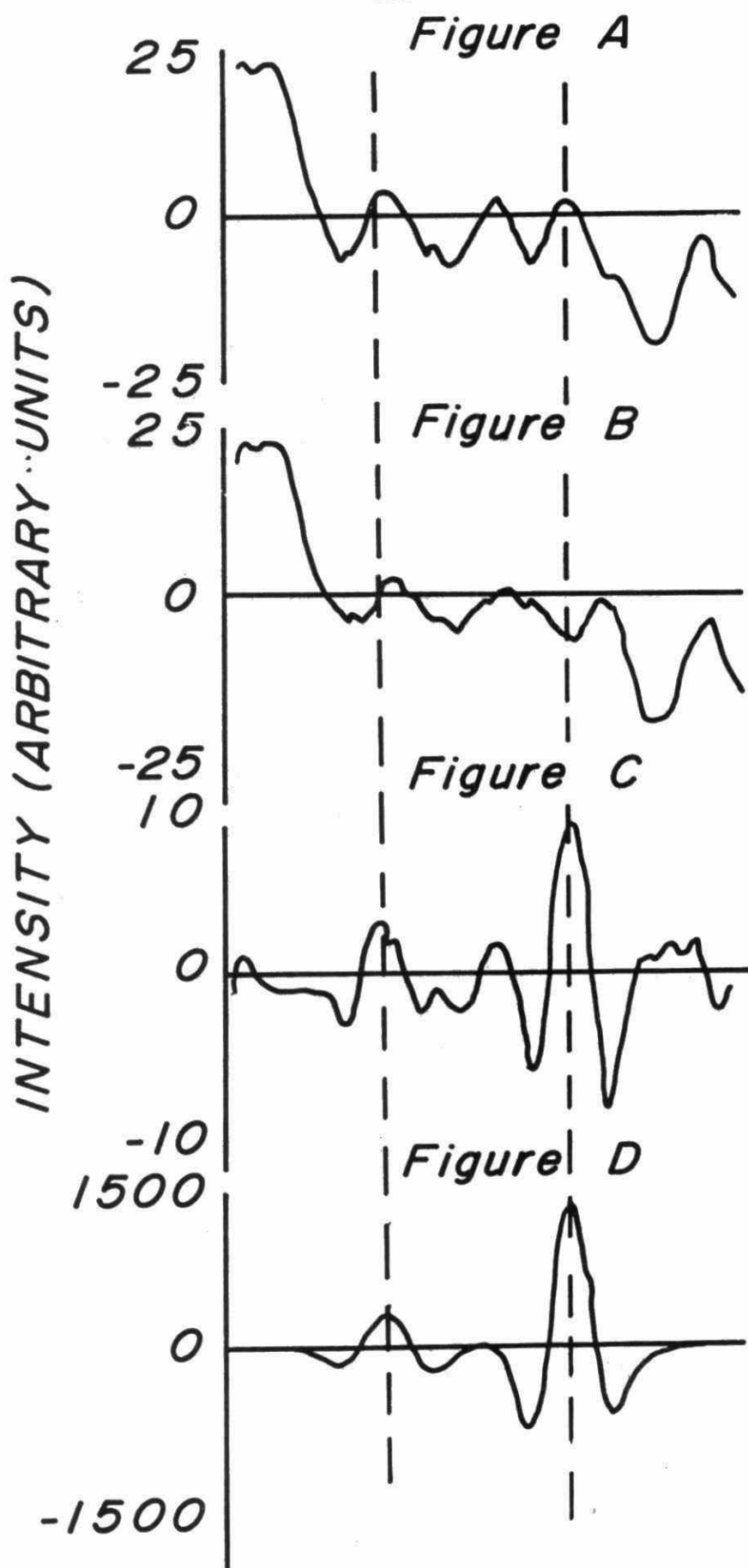
FIGURE 4. COMPUTER ASSISTED H_2O_2 DETECTION. A: 3.9 ppb H_2O_2 IN AIR; B: BACKGROUND. C: A-B; D: REFERENCE SHOWING THE H_2O_2 ABSORPTIONS.

The Dispersal of Airborne Particulates on a Short and Long Term Basis

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ABSTRACT

The environment receives many substances from anthropogenic and natural sources. In order to understand their extent and effect, many measurements of various types are necessary. Proton induced X-ray emission has been used to measure the element concentrations at the ng/m^3 level in air particulates collected by means of an air streak filter which provides temporal records on a short time scale of about an hour. Also concentrations have been measured at the ppm level in tree rings which provide a temporal record over many years.

Two species of pine trees at each of two locations, one near and downwind and the other relatively distant and upwind from Sudbury have been examined to determine the effect of the smelters in that city. No distinctive differences attributed to the installation of the high INCO stack in 1973 were observed. However, the cores of trees taken from near Sudbury showed increased concentrations for most of the elements, S, Cl, K, Ca, Mn, and Fe. When scans of the concentrations were measured with a beam of 25 μm, variations in the levels of several of the elements were observed during the growing season.

Streak filters were taken during a three week sampling period last winter at ground level some 50 m from a busy intersection in Kingston. Large variations in the levels of several elements were detected and these correlated highly with vehicular traffic. This was especially true for lead. Furthermore, Na, Cl and Ca levels were strongly correlated, suggesting a common origin which might be the sanding mixture since the residual fine road dust showed high levels of these elements. Daily averages for the concentrations observed inside and outside a school at the site were also measured.

That the quality of life depends significantly on the quality of the environment is becoming well recognized. Unfortunately, the environment is a dump, intentionally and unintentionally, for many wastes produced by society. In order to assess the extent of these wastes it is necessary to measure the concentrations of chemicals and elements as well as their movement through the environment. We have used proton induced X-ray emission (PIXE) and proton induced gamma ray emission to measure the element concentrations (1) tree rings in order to observe trends over many years and (2) of air particulates in order to observe the dispersal of automobile exhaust from a busy intersection.

AIR PARTICULATE PROJECT

Introduction

Air contains particulates, mostly between 0.1 and 10 μm in radius held in suspension by Brownian motion. The lower and upper limits are determined by coagulation and gravitational settling respectively. Understanding the source, transportation and removal mechanisms for these particulates is important since they may contain harmful substances, impair visibility and modify climates (Johansson et al., 1974)

Urban aerosols possess a bimodal mass distribution with gaussian peaks centred at 0.5 and 5.0 μm (Matsuo et al., 1978, Cahill et al., 1977, Whitby et al., 1972) which are characteristic of high-temperature combustion and natural sources respectively. Besides influencing the transportation of these aerosols their size is significant in determining where the particles are trapped in the human respiratory tract. The small particles, containing nearly all the toxic inorganic and organic materials, reach the pulmonary regions of the lungs (Natusch and Wallace, 1974).

A significant contribution to the smaller sized fraction of urban aerosols arises from automobile exhausts. Numerous studies of vehicle emissions have been conducted to characterize the size distribution (Martens et al., 1973; Paciga et al., 1975; Chan and Lawson, 1981), the chemical content (Habibi, 1973) and the dispersal (Chock, 1982; Bullen et al., 1982) of exhaust particulates. Considerable health hazards are associated with emitted lead salts resulting from the addition of tetraethyl lead to gasoline as an anti-knock

agent. Lead affects the central nervous system causing learning and
behavioral problems in young children (Marshall, 1982). Estimates show that
50% of the lead absorbed by the body (∿23 μg/day) is inhaled (Goldsmith and
Friberg, 1976) with 30% of the lead in the blood originating from gasoline
(McGinty, 1982). A direct correlation was observed between the drop in blood
lead levels and the 30% decrease in lead used for gasoline in the U.S.
between 1976 and 1980 (NHANES, 1976-1980).

In an attempt to assess the contribution which proton induced X-ray
emission could make to a study of air particulates, a streak air filtering
system was used to sample air near a busy intersection in Kingston. In
addition, its performance has been compared to a dichotomous sampler in a
joint study with the Kingston office of the Ontario Ministry of the Environ-
ment.

Experimentation

The air streak filtering system (Jensen and Nelson, 1974; Barfoot et al.,
1979) was housed in a rectangular box 0.6x0.2x0.2 m^3 in size, open on the
long sides to admit air through a coarse mesh. This box was mounted, 1.5 m
from ground level and air samples were collected at a site approximately
100 m SSW of a busy intersection (38,000 cars/day) and approximately 3 m from
a two story school for five weeks in Dec. 82 and Jan. 83. The air streaks
were collected on 0.4 μm Nucleopore moving at 1.0 mm/hr past a 2x2 mm^2 nozzle
through which air was drawn at 0.15 1/min. This flow was observed to be
constant within 10% for the duration of the sampling time. A simple calcul-
ation suggests that with this flow rate, all particles less than ∿30 μm would
be collected.

When it became clear that visible deposits were being collected at times
of high traffic density, the study was expanded by placing a second sampler
inside the school in order to compare the air inside and outside the building.
Since considerably less material was expected inside the school (an assump-
tion which proved to be false), only daily samples were collected on 0.4 μm
Nucleopore rather than on extended streaks.

Many studies (Watson et al., 1981) have shown that different filtering
devices produce particulate samples with different size characteristics.
Since the Ontario Ministry of the Environment has chosen the dichotomous

sampler, which collects fine and coarse fractions separately, to conduct a preliminary survey of the air quality throughout Ontario, the streak and the dichotomous samplers were run simultaneously in the same place so that a comparison could be made. The site chosen was atop the four-story Ontario Government Building in Kingston. During this comparison, the filters in the dichotomous sampler were changed every two hours over a twenty four hour period to match the two-hour resolution of the streak sampler.

All the filters were analyzed for the elements present using an external beam of protons from the Queen's University Van de Graaff accelerator and the technique of proton induced X-ray emission (PIXE). In this technique, protons of a few MeV energy bombard the sample dislodging electrons from the constituent atoms. These atoms emit X-rays which are characteristic of the atom. The X-rays are detected by a Si(Li) detector which produces a spectrum of the number of times an X-ray with a specific energy is observed. A typical spectrum from a spot on an air streak deposit is shown in Fig. 1.

The deposits from the air samplers are thin in the sense that the protons pass through the deposit losing relatively little energy (~100 keV). Hence the cross section for exciting the atoms is essentially constant and the self absorption of the X-rays is quite small. The number of X-rays coming from a specific element, x, may be written;

$$N_x = N_p \; n_x \; \Delta t \; \sigma x(E) \; \omega x \; \varepsilon_x \qquad\qquad A$$

where N_p is the number of incident protons,

$\sigma_x(E)$ is the cross section for dislodging an electron from element, x, when the energy of the proton is E,

ω_x is the probability that an X-ray is emitted when the atom relaxes,

ε_x is the detection efficiency for the detection of the X-ray produced,

n_x is the number density of the element,

and Δt is the sample thickness.

The quantities N_p, $\sigma_x(E)$, ω_x and ε_x are determined experimentally by placing a layer of an element of known thickness in the beam at the same spot as the sample and measuring N_x. Hence the system is calibrated and $n_x \Delta t$,

the areal density, for each element of the deposit is determined. Then, since the total area of the deposit as well as the flow rate is known, the amount of the element in the air can be determined.

Results

Fig. 2 is a photograph of a one-week streak with the Pb levels, precipitation and wind direction plotted on the same time scale. Strong correlation between the Pb levels and the dark deposits is evident. These deposits are in turn related to the periods of heavy traffic at the intersection, except when the winds were not blowing towards the site from the intersection. Precipitation does not seem to significantly affect the lead levels. In another test, measurements were made on the amount of lead in the road dust with a null result. Consequently, the automobile exhausts must be the source of this pollutant.

Time scans for peak areas of several other elements are shown in Fig. 3. Certain scans, e.g. S, appear unrelated to all other elements implying that S has a unique source. However, correlation is obvious for some of the elements, e.g. Cl, Ca and Na, thereby suggesting a common source. Fig. 4 is a scatter plot for Ca and Cl. These two elements are seen to be interdependent with a correlation coefficient of 0.88. Moreover, Cl, Ca and Na have high concentrations on Sat and Wed during rush hours, indicating their common origin is dependent on traffic. It is hypothesized that this source is road salt. If $CaCl_2$ and $NaCl$ were in equal proportions in the salt, the atomic ratio of Ca:Cl should be 1:3. Table I lists the concentration values for the major elements detected in Kingston air and compares these levels with those measured in other locations. From the Ca and Cl levels it can be determined that the measured atomic ratio is about 2.7, not inconsistent with the above assumption for the salt mixture.

Table II shows the results of the study in which 24-hour samples of the air particulates inside and outside the school were studied. For the two days studied, Table II shows that the concentrations of most elements were comparable inside and outside the school. The Nucleopore used in this study was free of Br; hence the Br/Pb ratio in Table II may be used to conclude that the source of the lead is gasoline since that ratio is within the range observed for gasoline exhaust (Paciga et el., 1975).

A comparison of the results of the streak and dichotomous samplers is presented in Table III. The concentrations are averages for the set of two-hour samples taken. The results for the individual two-hour samples are not presented because they are the same as for the average except that they are of lower statistical accuracy. An examination of the numbers reveals that, in general, the streak values approximate those for the fine fraction of the dichotomous sampler. The values for Ca and Al are somewhat higher indicating that some of the coarse fraction of the air particulates containing these elements is collected on the air streak deposit. Consequently the results from the streak sampler should be a good indicator of the air particulates which are likely to lodge in the lungs. Lead was not detected at this elevated site (<0.05 µg/m3). This seems surprising since the site near the busy intersection can be seen from this site and there are intervening roads and an adjacent shopping center where the traffic density is high. However, the streak sampler did reveal two instances of high lead concentrations (1.6 and 2.1 µg/m3), both in the middle of the night when the dichotomous sampler was not operating. Bromine was not detected at the same time which indicates that these high lead levels were not due to automobiles. Their cause remains unexplained.

Conclusions

An air streak sampler has been shown to be useful for observing changes in lead levels in air due to variations in traffic. Furthermore, the technique can monitor the level of other elements from other sources. The quantity of the air particulates inside and outside a building have been shown to be similar. However, the time variation of this correlation has yet to be established.

In comparison to a dichotomous sampler, it appears that the streak sampler collects the fraction of air particulates which appear in the fine stream of the dichotomous sampler. Consequently, streak samples can reveal time variations in the presence of air particulates that are likely to reach the lungs.

TREE RING PROJECT

Introduction

Many minor and trace elements have been observed in measurable quanti-
ties in trees (Meyer and Langway). Furthermore Hacskaylo et al. (1969), in
studying the nutritional requirements, has indicated that other elements
should be present in trees. Studies such as that of Thompson (1981) have
shown that the presence of pollutants, due to a nearby smelter, will influ-
ence the width of tree rings. That variations in the elemental concen-
trations might record the pollution in the environment was suggested by
Valkovic et al. (1979) and Lepp (1975).

A heartwood-sapwood differentiation in the concentration of chlorine was
observed by Tout et al. (1977) using neutron activation analysis. Several
other studies (Robitaille, 1981; Pillay, 1976; Baes III and Ragsdale, 1981)
using values from every fifth year or five year averages have shown that the
level of metals e.g. Pb, Cu and Zn, are elevated near smelters and roads
carrying a high traffic density, indicating that pollution in the environment
may be being recorded. However, large variations in the concentrations were
observed suggesting that the process of element deposition in trees is
complex. Consequently, the present study of the elements in tree rings was
undertaken with the external proton beam from the Queen's microprobe
(MacArthur et al., 1981) because it has a spacial resolution less than the
width of tree rings.

Experimentation

Since variations in element concentrations in trees were being sought,
cores were obtained from trees at several locations at various distances and
directions from the nickel smelters in Sudbury. It is well known that this
region shows the effects of pollution (LeBlanc et al., 1972). Consequently,
it was felt that variations in levels of the different elements might be
observed for trees from this region, especially since their environmental
conditions were supposedly changed with the introduction of the high INCO
stack in 1972. Two 1/4" radial cores were taken from each tree, one at stump
height i.e. 0.5m from the ground and one at chest height i.e. 1.5m from the
ground. These cores were mounted in teflon blocks and flat surfaces were

prepared by filing. Pictures of these surfaces are shown in Fig. 5. This preparation requires care because surface roughness as small as 10 μm can produce differences in counting rates due to the absorption of the X-rays. This is especially true when the detectors do not view the surface normally. Microscopic examination of the surfaces indicated that the roughness was less than a few μm. These samples were then mounted on a microstage and moved past the proton beam in steps as small as 80 μm.

These tree ring samples are thick samples, unlike the air streak deposits because the protons stop in the wood. Consequently, to find the number of X-rays observed, expression A must be integrated over the range of the proton as it travels through the wood. The counting rate observed is

$$N_x = \varepsilon_x \ \varepsilon x \ N_p \int_o^T \sigma_x(E) \ n_x(t) \ a(t) \ dt$$

where a(t) is the absorption of the X-rays from the depth t

and   T is the depth to which the proton penetrates before it stops. Protons and other charged particles lose energy at a known rate as they travel through matter i.e. dE/dt = S(E) where S(E) is the stopping power of the material, in this case wood, as a function of proton energy, E. Therefore

$$N_x = \omega_x \ \varepsilon_x \ N_p \ n_x \int_o^T \sigma_x(E) \ a(E) \ dE/S(E)$$

if n_x is assumed constant along the path of the proton.

Hence, the number of X-rays observed is independent of the thickness and density. The calibration of the system can be accomplished with substances of essentially the same basic composition as wood and which contain known quantities of elements likely to be found in wood. Since the sensitivity varies smoothly from one element to the next, the system does not have to be calibrated for every element expected.

The calibration was carried out with the following standards from the National Bureau of Standards - orchard leaves, pine needles and bovine liver. Two comments should be made about this calibration procedure. Firstly, the beam was moved on the sample in order to approximate the recommended sample size of 250-500 mg necessary for a reliable calibration using these standards. Secondly, it is necessary to assume that the concentrations of H, C and O and possibly N are similar in the standards and in the tree rings so that S(E) is

similar in the two cases. This is not a stringent requirement since C, O and N have roughly the same stopping powers. Therefore, protons will penetrate to the same depth in samples with similar proportions of H, C, N and O, such as the NBS standards and the wood samples.

Results

Several findings about the usefulness of PIXE and tree rings in the study of the distribution of elements in time have emerged from this investigation.

Fig. 6 shows the results for two of the many scans in the radial direction observed to date. Both scans are for the element Ca, one being for a core from a red pine on Levesque St. in Sudbury and the other for a core from a white pine at Penage. The two detectors on opposite sides of the beam observed the same variations at the same position which would not be the case if roughness were masking one detector and then the other. Consequently, these variations together with the fact that the counting rates are related directly to the concentrations, as was pointed out earlier, means that variations in concentrations of the elements are being observed.

To investigate how localized these changes might be, two scans 0.5 mm apart were taken through the same section of the core. Very similar patterns were observed. Consequently, the observed variations are not localized on the scale of 100 μm but are distributed concentrically with the centre of the tree in a fashion similar to rings.

For the red pine, increases in Ca levels occur in the early spring each year. For the white pine, this correlation is not as evident. However, the ring pattern for this white pine was very indistinct. In fact, many of the marks on the figure indicate positions where very narrow lines of slightly different shade were observed. Consequently, whether all the rings are being observed or not is unclear. Other scans of cores from white pines showed very clear rings which were perfectly matched with the changes in the Ca level each growing season. Whether careful measurements of these changes can be used as a tree ring count is still uncertain. The figure shows scans for Ca only. Elements such as K and Mn showed essentially the same pattern as Ca while others such as Fe, Cu and S showed no pattern in which the variations were correlated with the seasons. On the other hand, metals often showed

high, quite-localized concentrations. Visual observation of these regions of
the core, never revealed any structure or speck which would account for these
elevated levels.

Fig. 7 shows addition scans to show what little evidence has been found
to indicate that there are long term trends in the levels of the elements.
The dates indicated on the figure were determined by counting rings from the
bark inward. Clearly if rings are missed, these dates are in error. Never-
theless, the commissioning of the high stack in 1972 did not immediately
influence the deposition of these elements. Furthermore, the concentrations
of Ca and Mn have fallen with time in both species of trees while the level
of Fe has remained constant.

Table IV presents average concentrations for the elements observed
routinely in the two types of trees. Trees both distant and near the
smelters have been analyzed. Although there is an indication that trees
nearby have higher concentrations than those further away, such a conclusion
can only be drawn after considerably more samples have been analyzed.

Conclusions

It has been shown that PIXE can reveal the presence of many elements
at quite low levels (a few ppm) in tree rings. Variations in the concen-
trations of some of these elements, particularly Ca, Mn and K, occur
throughout the annual growing period and these variations can possibly be
used to measure the annual growth pattern.

The influence of a major, point source of pollution on the level of trace
and minor elements in trees has probably been established. The level of these
elements did not change dramatically or suddenly when efforts were made to
reduce the amount of pollution from this point source although the level of
some of the elements has decreased since the change was effected.

TABLE I

Concentration (µg/m3) of the Elements in the Air

Site

Element	Kingston Maximum	Mean	Rural U.K. Mean	Mexico City Mean	St. Louis Mean
S	0.7	0.1		8.	4.4
Cl	5.	1.	2.	1.	0.08
Ca	2.2	0.5	0.6		0.11
Fe	0.5	0.08	0.13	3.6	0.22
Pb	0.7	0.2	0.04	2.5	0.72

TABLE II

Average daily concentrations (µg/m3) found inside and outside the school

Element	Monday, Jan. 31 Inside	Outside	Tuesday, Feb. 1 Inside	Outside
S	6.0	1.8	1.3	1.2
Ca	0.41	1.3	0.91	5.7
Mn	0.038	0.050	0.047	0.13
Fe	0.13	0.25	0.20	1.0
Zn	0.059	0.043	0.054	0.078
Br	0.037	0.015	0.060	0.060
Pb	0.27	0.17	0.34	0.57

TABLE III

Element concentrations ($\mu g/m3$) in air compared for samples collected with the air streak sampler, with the fine and coarse stream of the dichotomous sampler

Element	Streak	Fine	Coarse
Al	0.83	0.25	1.7
Si	0.31	0.40	7.4
S	0.25	0.27	<0.05
Cl	0.40	0.50	8.4
Ca	1.70	0.23	7.1
Fe	0.20	0.15	2.8
Cu	0.03	<0.005	<0.01
Zn	0.012	0.05	0.07

TABLE IV

Concentration of elements in ppm for two species of trees comparing measurements taken on trees growing 8 km and 30 km from the smelter in Sudbury

Element	White Pine		Red Pine	
	Near	Far	Near	Far
S	608	615	200	80
Cl	210	139	171	139
K	730	500	810	550
Ca	1200	650	1520	990
Mn	50	25	100	250
Fe	100	65	60	30
Cu	17	14	35	19
Zn	26	22	35	29

References

C.F. Baes, III and H.L. Ragsdale (1981), "Age-Specific Lead Distribution in Xylem Rings of Three Tree Genera in Atlanta, Georgia," Environ. Pollut. B2, 21-35.

K.M. Barfoot, I.V. Mitchell, H.L. Eschbach, P.I. Mason, and W.B. Gilboy (1979) "The Analysis of Air Particulate Deposits Using 2 MeV Protons," J. Radioanal. Chem. 53, 225-271.

J.A. Bullen, N.J. Green and J.C. Polasek (1980), "Determination of Vehicle Emission Rates from Roadways by Mass Balance Techniques," Environ. Sci. and Tech. 14, 700-705.

T.A. Cahill, L.L. Ashbaugh, J.B. Barone, R.A. Eldred, P.J. Feeney, R.G. Flocchini, C. Goodart, D.J. Shadoan, and G.W. Wolfe (1977), "Analysis of Respirable Fractions in Atmospheric Particulates via Sequential Filtration," APCA Journal 27, 675-678.

T.L. Chan and D.R. Lawson (1981), "Characteristics of Cascade Impactors in Size Determinations of Diesel Particles," Atmos. Environ. 15, 1273-1279.

D. Chock (1982), "The Dispersion Analysis," Indus. Res. and Devel. Dec. 94-95.

J.R. Goldsmith and L.T. Friberg (1976), "Effects of Air Pollution on Human Health" in Air Pollution (3rd ed.), ed. by A.C Stern, Vol 2, 457-610.

K. Habibi (1973), "Characterization of Particulate Matter in Vehicle Exhaust," Environ. Sci. and Tech. 7, 223-234.

J. Hacskaylo, R.F. Finn and J.P. Vimmerstedt (1969), "Deficiency Symptons of Some Forest Trees," Research Bulletin 1015, Ohio Agricultural and Development Centre, Wooster, Ohio.

B. Jensen and J.W. Nelson (1974), "Novel Aerosol Sampling Apparatus for Elemental Analysis," in Nuclear Methods in Environmental Research, Conf. 740701, Oak Ridge, Tennessee, USERDA Technological Information Centre, 366.

T.B. Johansson, R.E. Van Greicken and J.W. Winchester (1974), "Interpretation of Aerosol Trace Metal Particle Size Distributions," Proc. of 2nd International Conference on Nuclear Methods in Environmental Research, ed. by J.R. Vogt and E.W. Meyer, 356-365.

F. LeBlanc, D.N. Rao and G. Comeau (1972), "The Epiphytic of Populus Balsamifera and its Significance as an Air Pollution Indicator in Sudbury, Ontario," Can. J. Bot. 50, 519-528.

N.W. Lepp (1975), "The Potential of Tree-Ring Analysis for Monitoring Heavy Metal Pollution Patterns," Environ. Pollut. 9, 49-61.

J.D. MacArthur, D. Amm, K.M. Barfoot, and M. Sayer (1981), "Materials Analysis with an External Beam Proton Microprobe," Nucl. Instr. Meth. 191, 204-210.

C.S. Martens, J.J. Werlowski, R. Kaifer, and W. John (1973), "Lead and Bromine Particle Size Distribution in the San Fransisco Bay Area," Atmos. Environ. 7, 905-914.

Y.M. Matsuo, M. Fujimura and Y. Hashimoto (1978), "A Proposal on Monitoring and Control of Aerosols Based on Size Distribution," in Environmental Systems, Planning, Design and Control, ed. by H. Aksahi and Sawargi, Pergamon Press, Oxford, 135-142.

L. McGinty (1982), "A Clear Case Against Lead in Petrol," New Scientist 94, 570.

J.A. Meyer and J.E. Langway (1973), "Nuclear Activation Analysis of Inorganic Elements in Wood," Wood Science 5, 270-280.

D.F.S. Natusch and J.R. Wallace (1974), "Urban Aerosol Toxicity; The Influence of Particle Size," Science 186, 695-699.

NHANES II (1976-1980) Second National Health and Nutritional Examination Survey, Vital Health and Statistics, PHS Publication no. 81-1317, Series 1, no. 15. Public Health Service, Washington, D.C.

J.J. Paciga, T.M. Roberts and R.E. Jervis (1975), "Particle Size Distribution of Lead, Bromine and Chlorine in Urban Industrial Aerosols," Environ. Sci. and Tech. 9, 1141-1144.

K.K.S. Pillay (1976), "Activation Analysis and Dendrochronology for Estimating Pollution Histories," J. Radioanal. Chem. 32, 151-171.

G. Robitaille (1981), "Heavy Metal Accumulation in the Annual Rings of Balsam Fir Abies Balsamea (L.) Mill," Environ. Pollut. B2, 193-202.

M.A. Thompson (1981), "Tree Rings and Air Pollution: A Case Study of Pinus Monophylla Growing in East-Central Nevada," Environ. Pollut. A26, 251-266.

R.E. Tout, W.B. Gilboy and N.M. Spryou (1977), "Neutron Activation Studies of Trace Elements in Tree Rings," J. Radioanal. Chem. 37, 705-715.

V. Valkovic, D. Rendic, E.G. Biegart, and E. Andrade (1979), "Trace Element Concentrations in Tree Rings as Indicators of Environmental Pollution," Environ. International 2, 27-32.

J.G. Watson, J.C. Chow and J.J. Shah (1981), "Analysis of Inhalable and Fine Particulate Matter Measurements," EPA-450/4-81-035

K.T. Whitby, R.B. Husar and B.Y.H. Liu (1972), "The Aerosol Size Distribution of Los Angeles Smog," J. Colloid. Interface Sci. 39, 177-211.
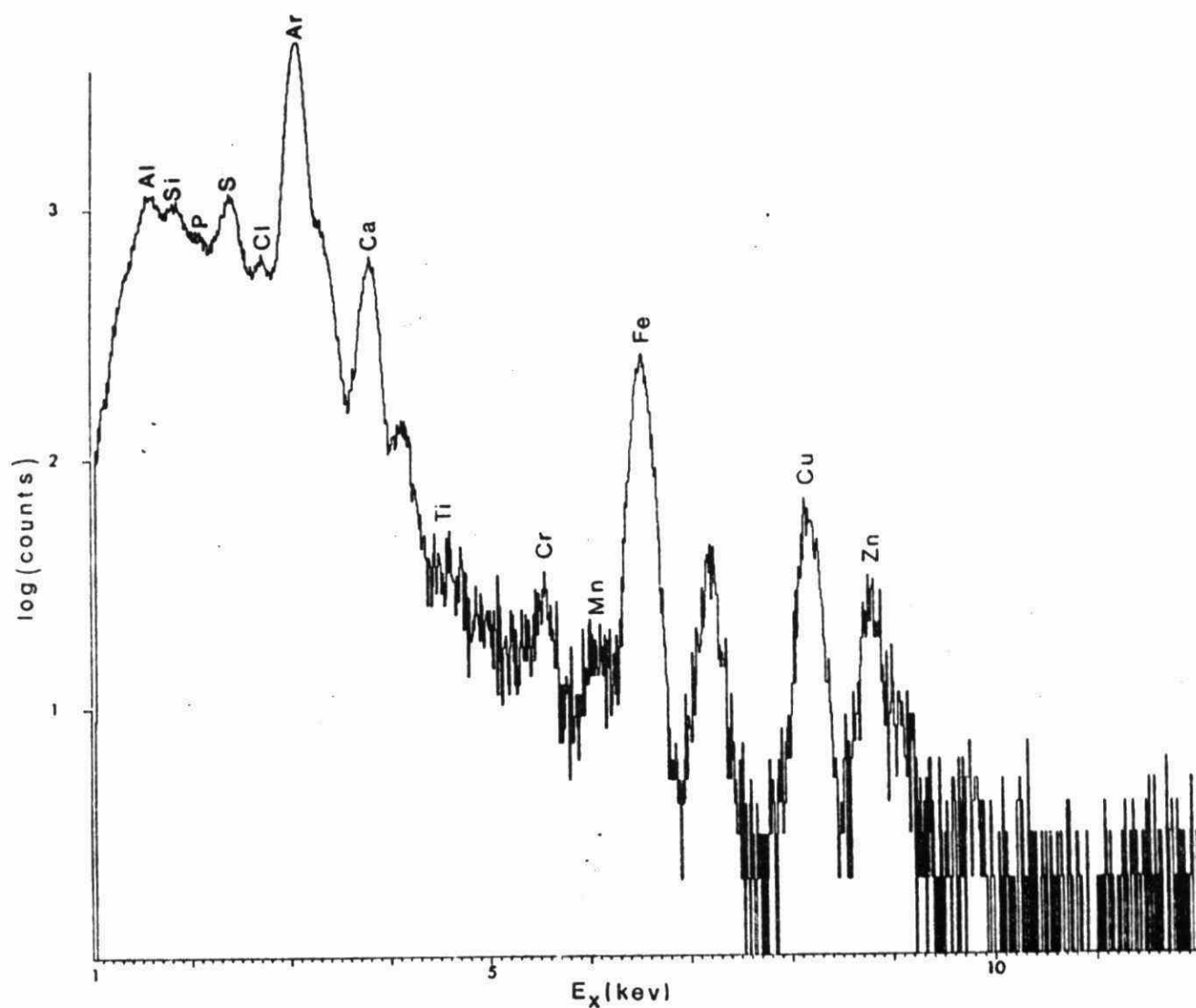
FIG. 1

An X-ray spectrum from an arbitrary spot on a streak sample taken on the
roof of the Ontario Government Building in Kingston. The sample was
excited in air with a proton beam of 2.5 MeV, hence the presence of a
strong Ar line. Unlike spectra observed from the streaks obtained at
ground level, this spectrum shows no sign of Pb at an energy of 10.5 keV.
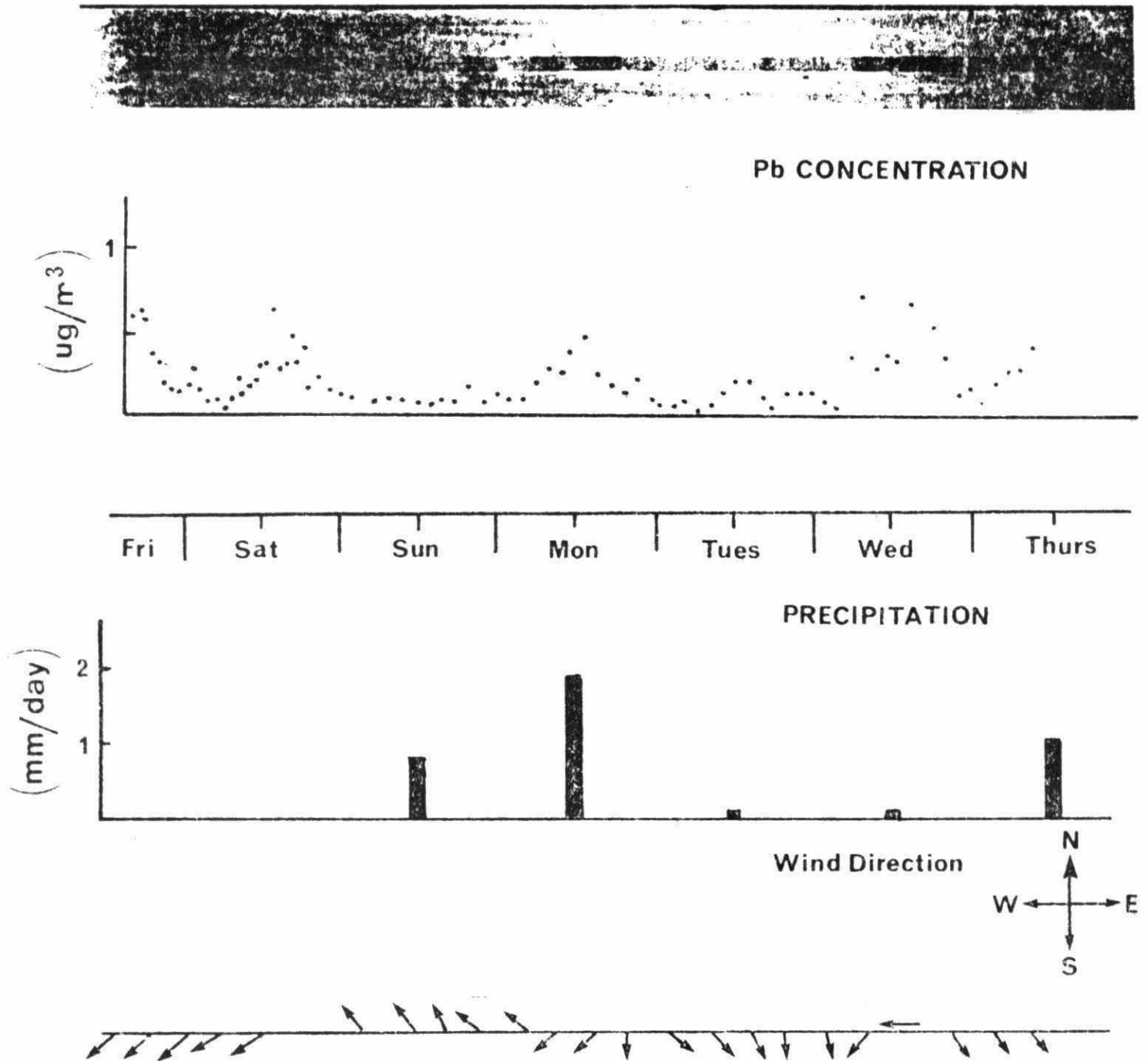
FIG. 2

A photograph of an actual streak together with meteorological data and a time profile of the lead concentration which is correlated with high traffic densities.
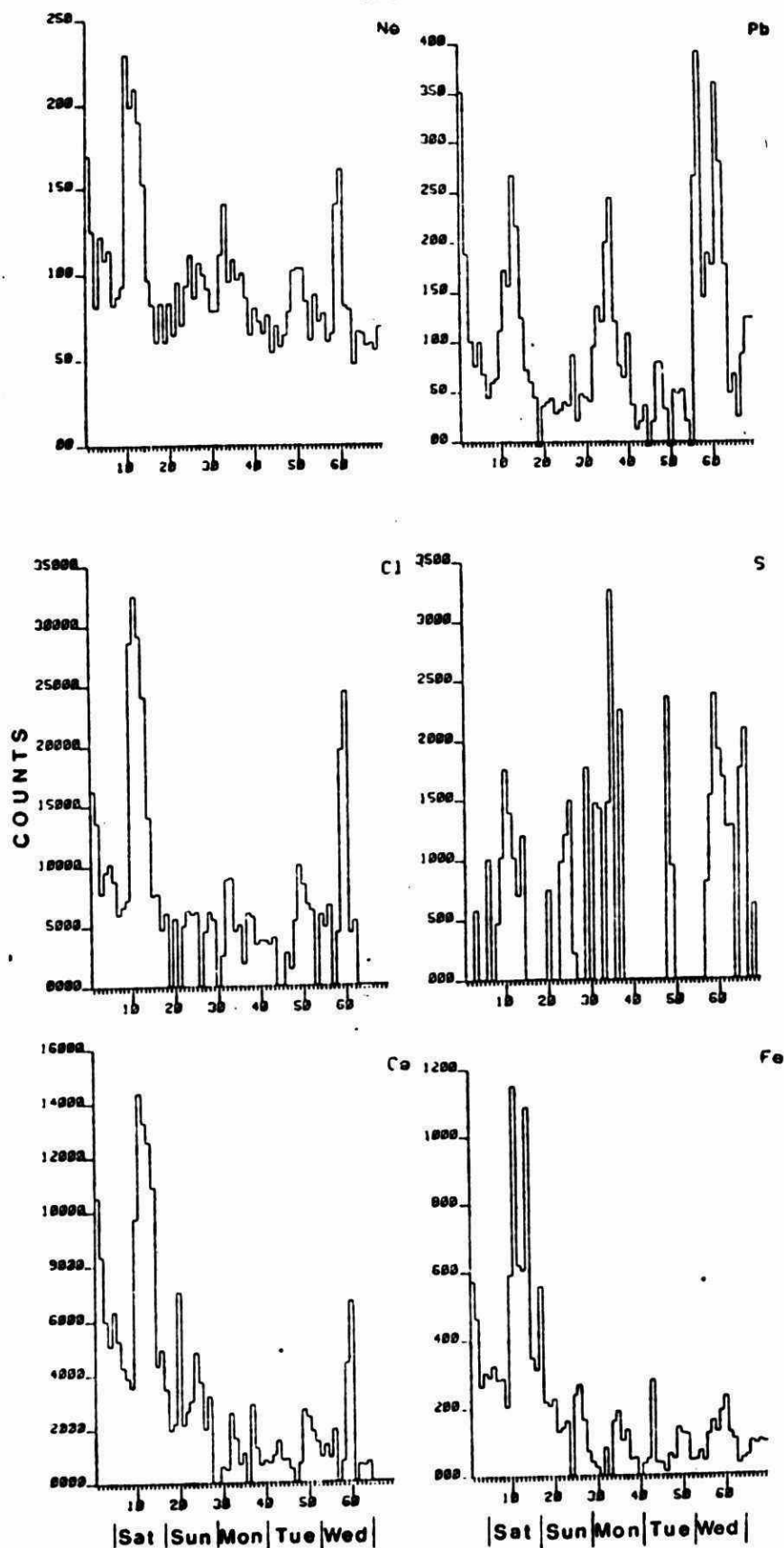
FIG. 3

Time scans for the elements detected in the
particulate matter trapped on the air filter
of Fig. 2. Each bar represents an average
over a two-hour period.
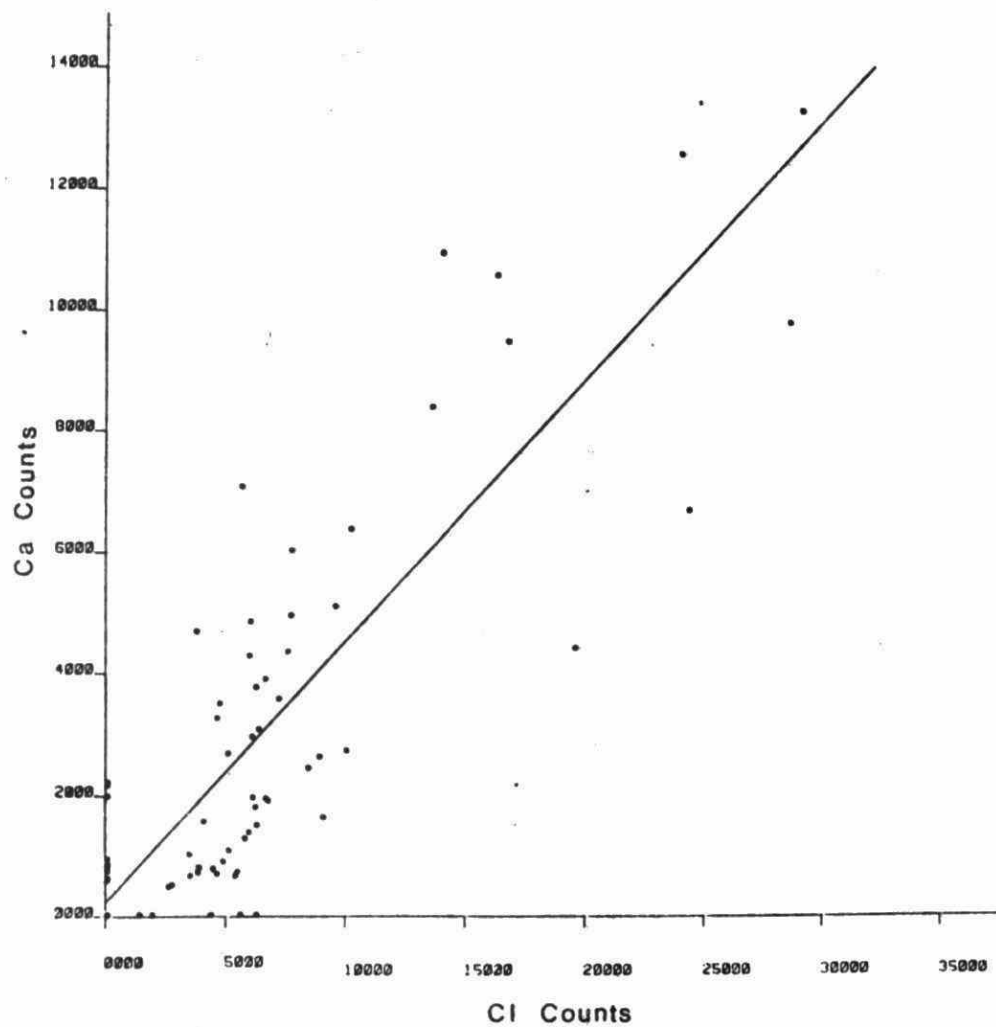
FIG. 4

A scatter diagram showing the high correlation (r = 0.88) between the Ca and the Cl concentrations observed in the streak of Fig. 2.
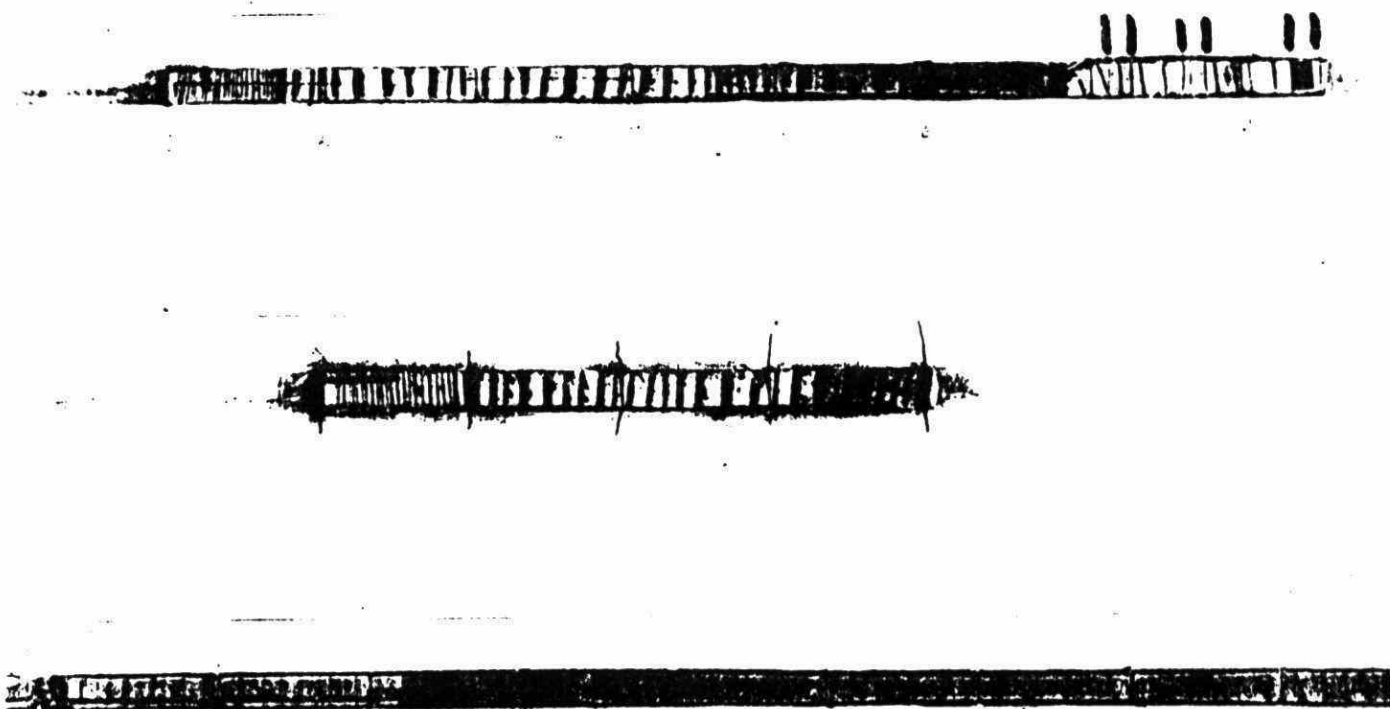
FIG. 5

Photographs of the core samples analyzed for their element concentrations with a 25 μm proton beam. The top two photographs are for red pine where the tree rings are quite distinct; the third one is for a white pine. The lines, approximately through the middle of the core, are an indication of where the beam hit the samples.
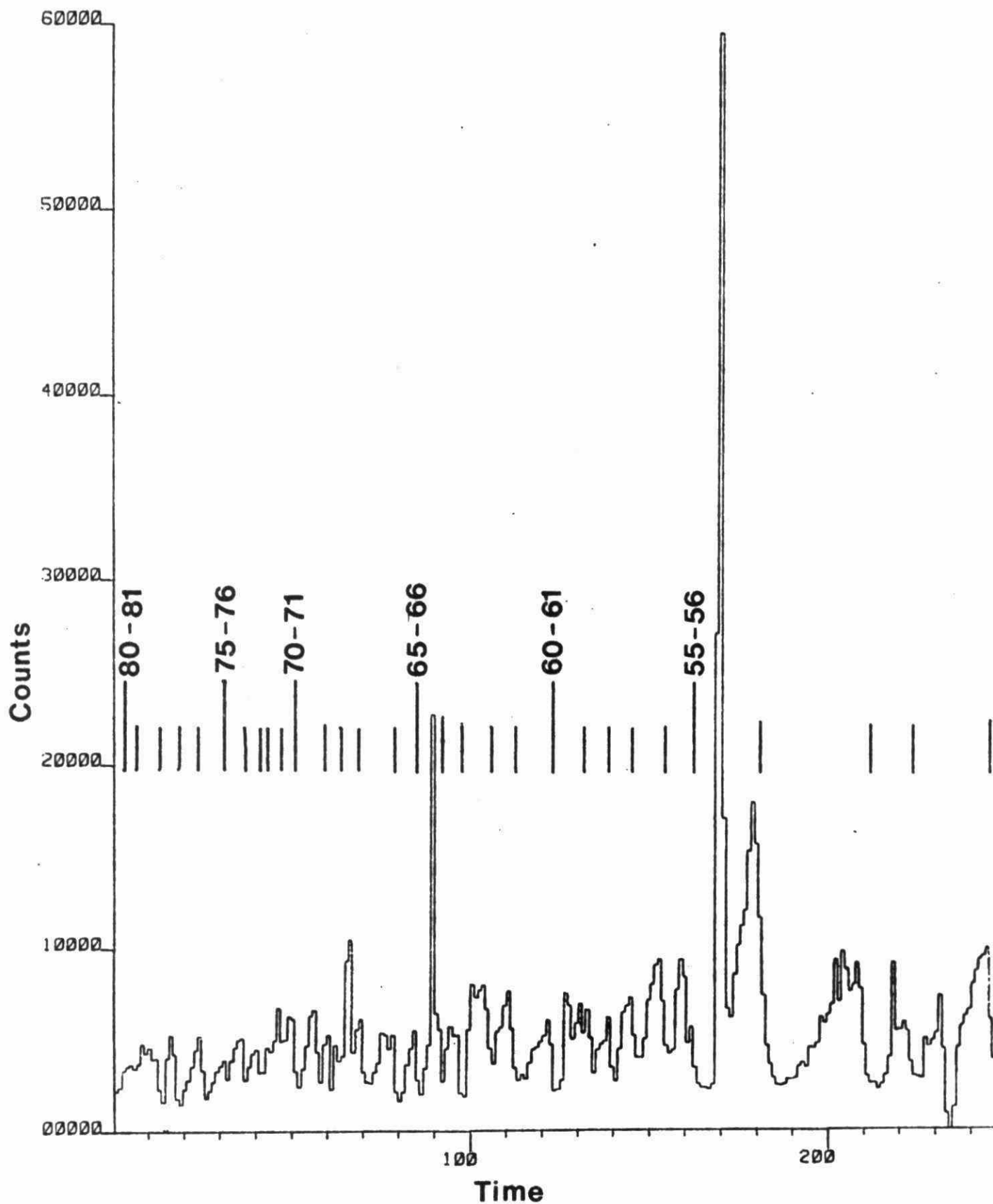
FIG. 6a

Time scans of the Ca counts in a sample of red pine. The lines added to the figure indicate the position of the dark rings observed visually. There is a distinct pattern in which the Ca counts rise in the spring.
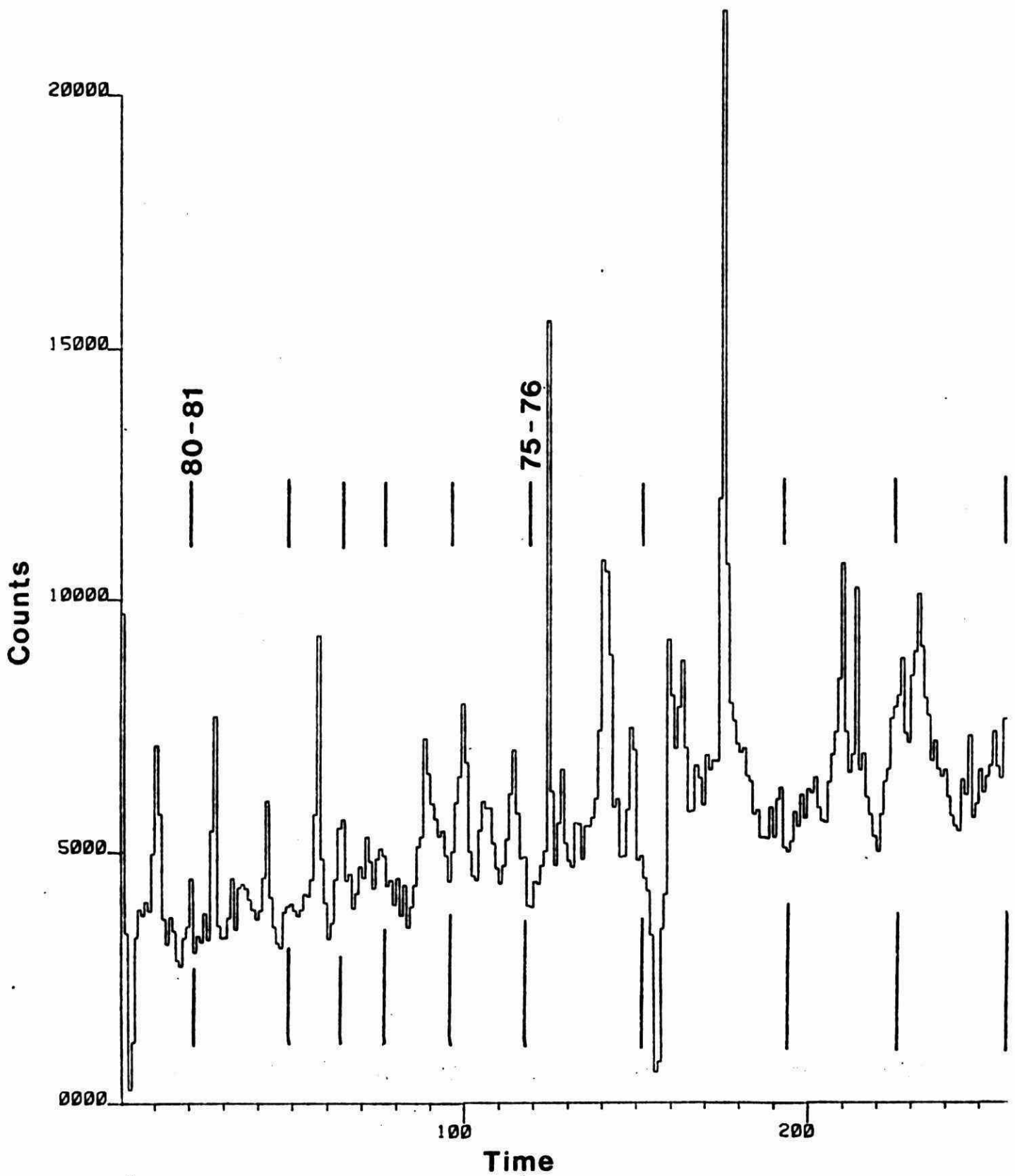
FIG. 6b

Time scans for a white pine similar to Fig. 6a. The Ca seasonal pattern is not obvious.
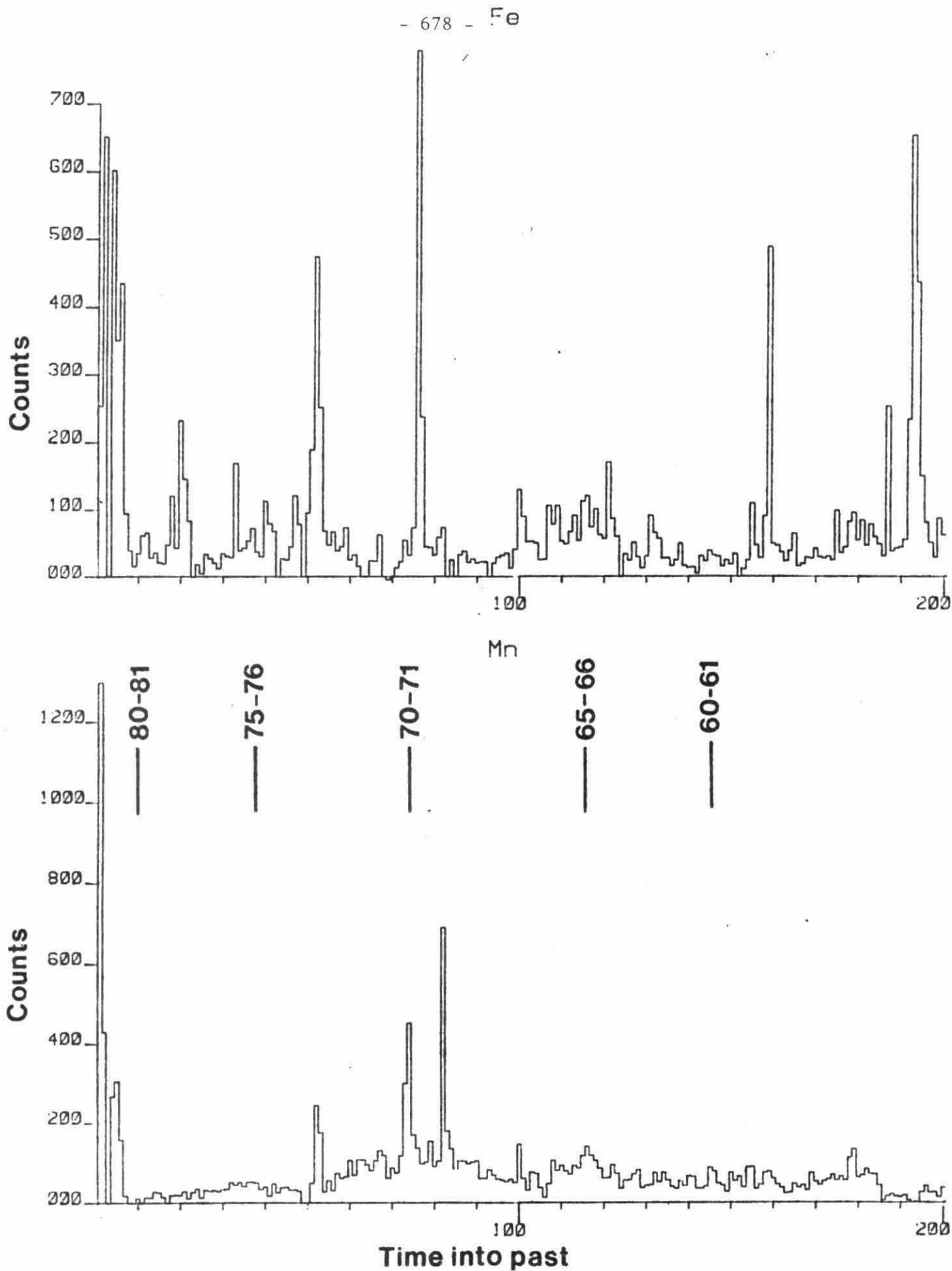
FIG. 7

Time scans for Fe and Mn counts in samples of white pine(a) and red pine(b)
taken near the smelters in Sudbury. After 1973, the concentrations of Ca
and Mn decrease while the Fe levels remain constant.
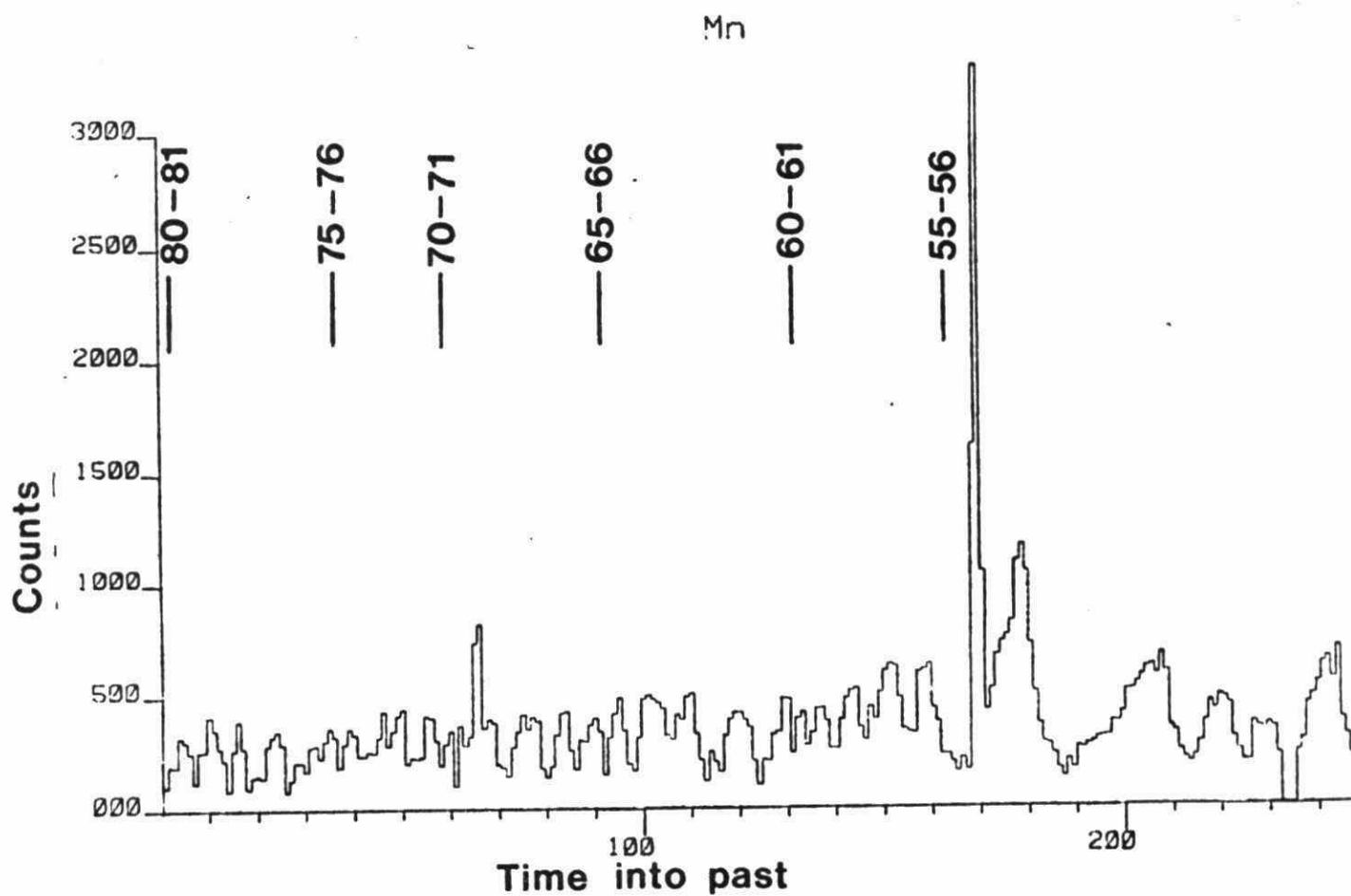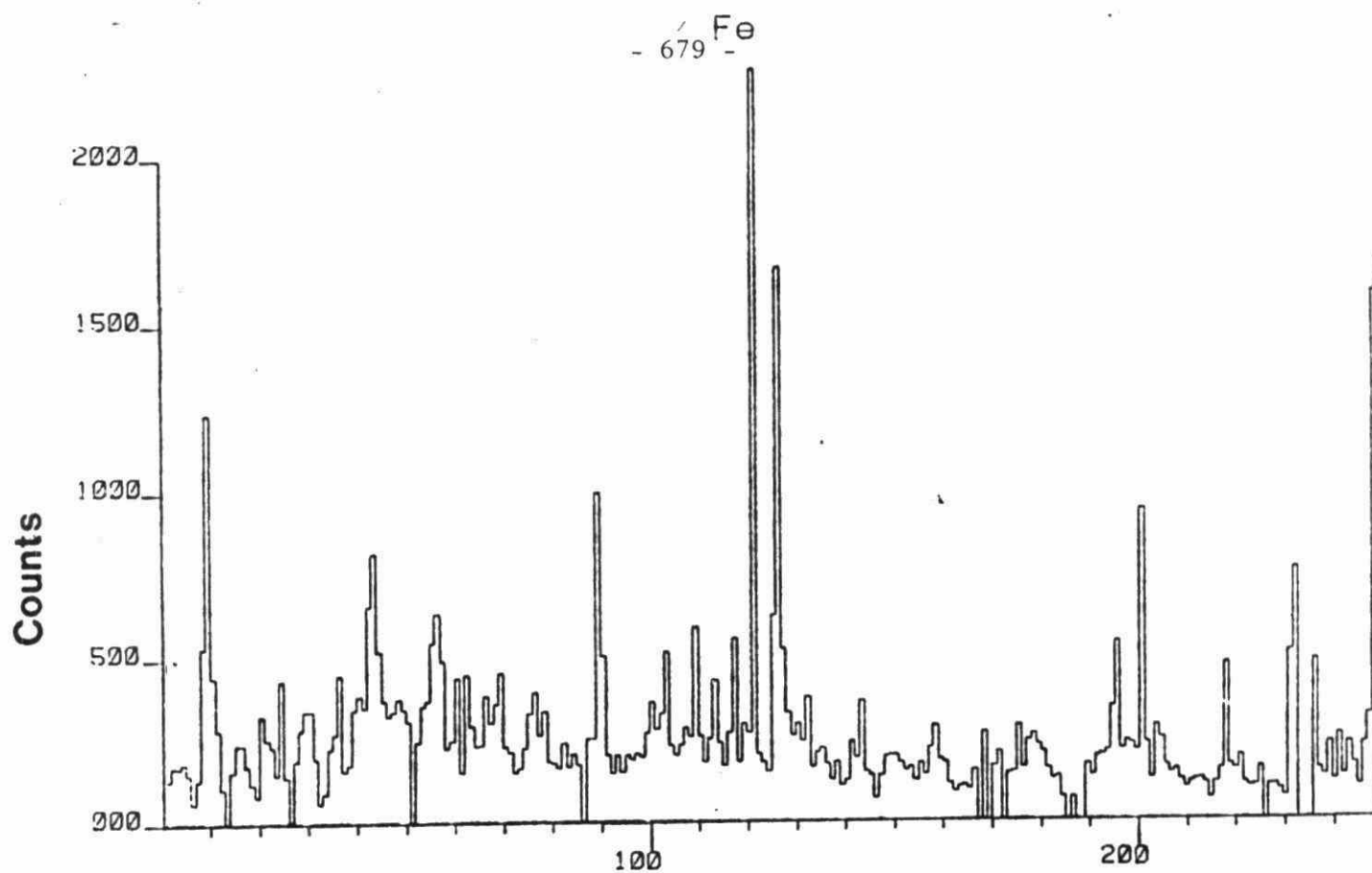
FIGURE 7(b)

Assessment of impact of oxidant injury on development of early blight on potato: implications for disease control.

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ABSTRACT:

Some cultivars of potato grown in Ontario are very sensitive to O_3 and can sustain a lot of foliar injury some years. Some of these same cultivars like Norland and Norchip are very sensitive to early blight Alternaria solani.

The symptoms produced by O_3 or early blight when they occur alone are fairly distinct. When the two agents occur together a different symptom is produced. The new symptom is the result of the fungus infecting the many small lesions produced by O_3.

Reducing O_3 injury on sensitive cultivars increases the control of early blight by fungicides. Initial experiments were conducted with the antioxidant EDU which is no longer available. In 1983 plots were set up at a Research Station and in growers fields to determine whether fungicides with antioxidant properties, in combination with a fungicide effective in controlling early blight, would give better disease control.

Ozone injury and disease incidence were minimal until about mid-August. After that time considerable injury from both agents occurred. Because of the relative lateness in the season only two plots were still available for assessment. The effect of the fungicide manzate, which has antioxidant properties, and the effective fungicide, DuTer, applied singly or in combination, on foliar injury and yield will be discussed.

Introduction

In some crops yield suppression by ozone is directly related to O_3 injury on the leaves. In other crops the increased susceptibility to parasitic diseases due to O_3 injury appears to have the greater impact on yield. Over the last several years we have investigated the impact of O_3 on potato yield and the influence of O_3 injury on early blight epidemiology.

Sensitive cultivars like Norland have been deemed to be severely affected by O_3 but documented evidence is scarce. Several other cultivars also have a high degree of O_3 sensitivity (Table 1). Cultivars that are sensitive to O_3 also seem to be susceptible to early blight, suggesting a possible interaction between the two agents.

Experiments have been conducted (1) to determine the relative sensitivity to O_3 various potato cultivars in Ontario; (2) to distinguish between O_3 symptoms and early blight symptoms; (3) to determine the impact of O_3 in potato yield; (4) to establish whether an O_3/early blight interaction occurs and its significance; (5) to work out the implications of the interaction on disease control programs.

Experimental procedures

Most of the work has been conducted in the field in various parts of Ontario, backed up by lab studies as required. Plots were established in growers' fields or at experimental stations. The approach used, was to control one or both agents by means of chemicals, and to assess injury development over the growing season. While available the antioxidant EDU was used to suppress O_3 injury and the most effective fungicide - Du-Ter to control early blight. During the 1983 growing season only fungicides were used: manzate to suppress O_3 injury and disease development; Du-Ter to control disease; or the formulated mixture of Manzate and Du-Ter (similar to Liromatin).

From the beginning of July to the end of August spray applications were made every two weeks. Foliar injury was assessed weekly and symptoms differentiated between O_3 stipple, expanding blight lesions and the interaction symptoms. At the end of the season potatoes were harvested, and yield and specific gravity determined.

Results and Discussion

Over the last 4 years the amount of O_3 injury has been small on sensitive cultivars like Norchip. In 1983 even the very sensitive cultivar Norland, which can be completely defoliated by O_3 by late July, showed no injury until mid-August. In none of the last 4 years could O_3 injury be considered as severe. Eventhough EDU suppressed O_3 injury on potato, only

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infrequently did it result in yield increases in the sensitive cultivar Norchip. The overall effect over 3 years was not significant (Table 2).

Reduction in O_3 injury by EDU frequently led to better control of early blight by the fungicide Du-Ter. Disease development was suppressed and yield and specific gravity enhanced (Table 3). Normally early blight shows up on potato leaves as a few large lesions which expand with time. When O_3 injury is first present many small blight lesions develop on the leaf, indicating that O_3 lesions become quickly colonized by the early blight pathogen.

That fact that an antioxidant in combination with a good fungicide gave better control of early blight, led to an assessment of available chemicals for more effective control. Manzate a chemical used for early blight control has been known for some time to have antioxidant properties. Du-Ter in our tests has been shown to be the most effective fungicide against early blight. A special formulation consisting of a mixture of these chemicals was made up for test purposes in 1983.

Initially 4 locations in Ontario were selected for testing: Grand Bend, Carlyle, Arkell and Alliston. Because of drought and early harvest by the grower, the plots at Grand Bend developed no O_3 injury and disease symptoms. The plots at Carlyle had insect and wilt problems and were also abandoned. The Alliston and Arkell plots were very dry most of the season resulting in low yields as in the commercially grown potatoes. O_3 injury became first apparent on August 17, but never consisted of more than 6% injury on Norland at Arkell and 30% at Alliston. Manzate significantly reduced O_3 injury (Table 4). With the longer nights and longer dew periods of late August early blight developed rapidly soon after O_3 injury was seen.

At the early stages of the disease Manzate appeared to give somewhat better control than Du-Ter, whereas once the disease progressed rapidly, Du-Ter and Liromatin gave better control (Table 4). It appears that if O_3 injury occurs before the early blight epidemic occurs a compound with antioxidant properties gives somewhat better control, presumably though a reduction in O_3 lesions. Once the disease is established the compound with the best fungicidal properties gives better control. The combination treatment (Liromatin) seemed to be the most effective in disease control, although often not statistically better than Du-Ter.

Yields were low with drought being the overriding faster. However, effects on yield of the treatments were still apparent in Norchip (Table 6). The different chemical treatments were not significantly different from each other for the 1983 season.

Summary

Drought, low levels of O_3 injury which occurred late, and rapid disease development in late August 1983 minimized differences between treatments.

Manzate reduced O_3 injury and appeared to give better disease control at the beginning of the epidemic.

The mix tended to give better disease control overall.

Reducing O_3 injury delays the onset of early blight epidemic. Therfore

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a fungicide with antioxidant properties is more effective early in the season. The fungicide most effective against the disease should be used once the epidemic has become establsihed.

Acknowledgement

Publications arising from the project

Hofstra, G., R.T. Wukasch and D.M. Drexler. 1983. Ozone injury on potato foliage as influenced by the antioxidant EDU and sulphur dioxide. Can. J. Plant Pathol. 5:115-119.

Holley, J.D., R. Hall and G. Hofstra. 1983. Identification of rate-reducing resistance to early blight in potato. Can. J. Plant Pathol. 5:111-114.

Holley, J.D., G. Hofstra and R. Hall. Effects of ozone and early blight on foliar necrosis and tuber yield of potato. Phytopathol. In preparation.

Holley, J.D., R. Hall and G. Hofstra. Effect of leaf wetness and temperature on the rates of development of early blight on three potato cultivars. Can. J. Plant Pathol. In preparation.

Holley, J.D., G. Hofstra and R. Hall. The effect of oxidant injury on the appearance, fine structure, and development of early blight lesions on leaves of "Norchip" potato grown in the field. Can. J. Plant Pathol. In preparation.

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Table 1:   The relative O_3 sensitivity of various potato cultivars.

| Cultivars | % Ozone injury Dates of Observation | |
	6/8/81	25/8/81
Norland	35.0	90.0
Campbell 13	21.0	50.0
Cherokee	25.0	40.0
Bel Rus	6.0	35.0
Monona	10.0	35.0
Norchip	9.0	30.0
Chieftain	8.0	25.0
Yukon Gold	12.0	20.0
Trent	7.0	20.0
Katadhin	9.0	12.0
Atlantic	6.0	8.0
Sebago	3.0	7.0
Superior	3.0	−
Rideau	1.0	7.0
Russet Burbank	0	3.0
Kennebec	0	2.0

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Table 2: The effect of EDU, Du-Ter singly or in combination on yield in Norchip.

Location	Relative Row Weights			
	Check	EDU	Du-Ter	EDU + Du-Ter
Alliston 1980	19.50	20.90	22.00	23.90
Alliston 1981	14.00	12.50	16.00	17.50
Carlisle 1981	-	-	-	-
Alliston 1982	17.80	18.70	19.80	20.20
Burlington 1982	17.40	17.10	18.70	21.40
Means	17.20x	17.30x	19.10y	20.80y

LSD test at $P = 0.05$.

Table 3. The effect of EDU, Du-Ter singly or in combination on yield, specific gravity and infection rate on 3 cultivars (Norchip, Chieftain and Kennebec) over 3 years.

Treatment	Row Weight	Specific Gravity	Apparent Infection Rate
Check	20.38a	1.0686d	0.172g
EDU	19.97a	1.0689d	0.168g
Du-Ter	22.32b	1.0709e	0.148h
Du-Ter + EDU	22.63b	1.0716f	0.146h

LSD tests at P = 0.05

Table 4: Maximum observed ozone injury on Norland and Norchip potato at Arkell and Alliston, 1983.

	Norland	Norchip
Arkell	(August 22)	
Control	3.6 ab	2.1 a
Manzate	2.1 b	0.9 a
Du-Ter	6.3 a	1.0 a
Mix	4.0 ab	0.9 a
Alliston	(August 23)	
Control	31.9 a	5.5 a
Manzate	11.9 b	1.5 b
Du-Ter	20.0 ab	4.2 a
Mix	15.5 b	2.3 b

Differences significant at $P = 0.05$.

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Table 5: Disease ratings partway through the early blight epidemic on
Norland and Norchip potato at Arkell and Alliston, 1983.

	Norland	Norchip	
Arkell	(August 23)	(August 26)	(August 30)
Control	87.5 a	68.6 a	97.5 a
Manzate	39.0 b	17.4 b	80.0 b
Du-Ter	58.8 b	20.6 b	76.8 b
Mix	37.4 b	14.3 b	68.0 b
Alliston	(August 23)	(August 29)	(Sept. 5)
Control	87.5 a	11.3 a	66.9 a
Manzate	68.6 b	4.4 b	27.7 b
Du-Ter	71.2 b	4.4 b	15.8 c
Mix	64.2 b	4.6 b	13.5 c

Differences significant at P = 0.05

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Table 6. Yield of Norland and Norchip potato at Arkell and Alliston.

	Norland	Norchip
Arkell	kg/row	
Control	10.2 a	7.8 b
Manzate	10.8 a	9.9 a
Du-Ter	9.9 a	9.7 a
Mix	10.6 a	9.3 ab
Alliston		
Control	9.5 a	11.0 b
Manzate	10.5 a	12.8 ab
Du-Ter	10.1 a	13.3 a
Mix	10.5 a	13.3 a

Differences significant at P = 0.05.

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Production of Ozone Insensitive Field Bean Varieties

A presentation submitted to the Technology Transfer Conference No. 4,
Ontario Ministry of the Environment

T.E. MICHAELS, W.D. BEVERSDORF, B.D. MCKERSIE

Common bean (Phaseolus vulgaris L.) suffers foliar injury following episodes
of high atmospheric ozone concentration. Bronzing, the reddish-brown stippling
of the upper leaf surface is a symptom of the ozone injury. Bronzing is
associated with loss of photosynthetic area, leaf abscission, and premature plant
senescence.

White beans, also called navy or pea beans, are a common bean grown on
25,000 to 40,000 ha in southwestern Ontario. These beans are sold on Canadian
and foreign markets for canning, and represent cash receipts of $30 million to
Ontario farmers. Bronzing in white beans has been noted at ozone levels in
excess of 9 pphm. The plants appear most susceptible during pod fill in mid to
late August. Using the anti-oxidant ethylene diurea, G. Hofstra and others from
the Department of Environmental Biology at the University of Guelph have
estimated a 32% to 36% reduction in white bean yields as the result of ambient
ozone levels at Elora and Ridgetown.

Though field beans in general are sensitive to ozone, insensitive cultivars
have been found. These cultivars are of improper size, shape, color, or
environmental adaptation for direct use in Ontario white bean production.
However, this genetic variation in response to ozone can be exploited to develop
ozone insensitive white bean cultivars adapted to Ontario by intercrossing
insensitive with adapted, sensitive cultivars.

Experiments conducted by the Department of Crop Science at the University of
Guelph with funding from the Ontario Ministry of the Environment were designed to
identify genetic sources of ozone insensitivity, characterize the inheritance of

insensitivity, initiate breeding populations incorporating insensitive sources and evaluate the cultivars derived from these populations.

Controlled environment studies and field trials were conducted with 162 field bean cultivars and plant introduction lines in 1978 and 1979. Snap bean cultivars were found generally less sensitive to ozone than white bean cultivars. The wax bean Gold Crop showed outstanding insensitivity to ozone. French Horticultural, Calima and Narda also exhibited little sensitivity. All white bean cultivars currently grown in Ontario were sensitive. The insensitive lines were intercrossed with the adapted white bean cultivars Seafarer, Ex Rico 23, and Kentwood to form breeding populations for inheritance studies and cultivar selection. Inheritance studies suggested that ozone insensitivity is controlled by multiple nuclear genes. Heritability estimates from controlled environment studies were high (H = 0.66 to 0.88) while estimates from field trials were lower (H = 0.16 to 0.21). These heritabilities suggest that selection for ozone insensitivity should be carried out in later generations of self pollination when individual plants are nearing homozygosity, and parental performance adequately predicts performance of progeny. We have chosen to integrate selection for ozone insensitivity with selection in the field for quality, architectural and yield characteristics, since these attributes must also be evaluated in plants nearing homozygosity.

Successful selection in the field relies upon cooperative environmental conditions, in this case, high ambient ozone levels. Conducting selection trials in multiple locations and years improves the probability of success. In 1983, over 1000 plots at Elora, Woodstock, Ailsa Craig, Mitchell and Palmerston were evaluated for insensitivity. Foliar injury was most apparent at the Woodstock breeding nursery and Mitchell yield trial. Two advanced line trials involving

potential cultivars derived from Ex Rico 23 and Kentwood crosses with Narda were evaluated at Woodstock. Preliminary analyses indicate that most Ex Rico 23 x Narda derivatives were significantly less sensitive than Ex Rico 23. Kentwood was less sensitive than a Kentwood x Narda derivative (Table 1). These lines were selected for white bean quality, yield and disease resistance with little or no selection pressure against sensitivity. We would expect some lines not to surpass the parent for ozone insensitivity.

Integrated selection for quality, yield and ozone insensitivity was conducted this summer on 6 F_5 populations at Woodstock. The most insensitive lines from populations 79-1 (Narda x Eagle) and 79-2 (Narda x Provider) tended to show snap bean characteristics, but one population, 79-4 (Seafarer x Calima), contained two highly insensitive white bean type plants. Progeny of these plants will be evaluated and increased in 1984 for replicated quality, yield and insensitivity trials in 1985. Forty-five new F_5 populations will be evaluated for insensitivity in 1984, eight of which incorporate previously identified insensitive sources as parents.

Promising new sources of insensitivity were identified in the 1983 CIAT International Bean Flowering and Adaptation Nursery. The IBFAN was designed to assess the effect of latitude and climate on flowering, and included a wide range of germplasm. Five entries with early flowering dates and ozone insensitivity will be incorporated into the breeding program.

These initial efforts to improve ozone insensitivity of white beans constitute the first cycle of selection. First cycle selections can be inferior in quality or yield attributes if unfavorable germplasm is incorporated along with that conferring insensitivity. Second cycle selections, resulting from intercrossing lines selected in the first cycle, should show superior combinations of quality, yield and insensitivity.

Table 1. Ozone sensitivity of advanced breeding lines, Woodstock, Ontario, 1983.

Line	Background[1]	Rating[2]
W15-01	A	1.75
W07-01	B	2.00
W11-18	A	2.00
W13-03	A	2.25
W13-18	A	2.50
W07-03	B	2.50
W13-07	A	2.75
W15-19	A	3.00
W11-03	A	3.00
W11-02	A	3.25
W13-04	A	3.50
W07-08	C	3.75
W11-09	A	4.00
Seafarer		3.50
Kentwood		1.75
Ex Rico 23		3.75
$LSD_{0.05}$		0.75

1. A = Ex Rico 23 x Narda
   B = Ex Rico 23 x W77-08
   C = Kentwood x Narda

2. 0 = no bronzing, 5 = severe bronzing

Sweet Corn and Green and Wax Bean Responses to Air Pollution in Southern Ontario

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Abstract

Replicated field experiments were conducted in 1982 and 1983 at the University of Guelph-Ontario Ministry of Agriculture and Food Research Station at Cambridge, Ontario; at the Ontario Ministry of Agriculture and Food Horticultural Experiment Station at Simcoe; and at the Canada Department of Agriculture Research Station at Harrow. Randomized block split-plot experiments were conducted with three concentrations of benomyl as a chemical protectant spray or drench, applied to plants weekly. Appropriate benomyl concentrations were established in laboratory studies with sweet corn and from literature references for beans. There were six cultivars of sweet corn and of snap beans at each location. Tobacco ozone-indicator plants were placed in each plot. The 1982 harvest data indicated that while there were substantial differences among yields of cultivars at each location, the relative differences were generally similar at all locations. The few changes in cultivar yield patterns may have reflected differences in temperature, rainfall, and other environmental responses, as well as differences in sentivity to air pollution. There were few statistically significant effects of benomyl sprays in these studies. The effect of 4000 ppm sprays was injurious in some cases, particularly in the sweet corn experiment at

Simcoe. Yield increases with 1000 ppm benomyl sprays were noted in some corn cultivars at Cambridge, and generally in corn at Harrow and in beans at Cambridge. Additional measurements made at the sweet corn harvest indicated that the benomyl effect on husk weight was always similar to that on total yield while cob number was significantly increased by benomyl only at Cambridge and cob size increased only at Harrow. Data analyses and interpretation for the 1983 field experiments are not yet complete, and will be reported later.

Introduction

Southern Ontario crop production areas are subjected to recurring ozone (O_3) episodes during the growing season. While the responses of many crops to O_3 in the field in southern Ontario have had considerable study in the past, those of sweet corn and green and wax beans have had little attention. The commercial production of sweet corn and green beans for the fresh market or for freezing or canning is an important horticultural activity in Ontario. In addition, both species are widely grown in home gardens. Studies elsewhere have indicated that at least some cultivars of each species are sensitive to O_3. Experiments in the field in southern Ontario are necessary to permit evaluation of a range of cultivars of each species under the weather and pollution conditions characteristic of this area. An understanding of cultivar sensitivities, location effects, weather interactions, amelioration techniques and various other air quality relationships is essential for an understanding of pollution

limitations on productivity of these crops.

A study was initiated in 1982 to evaluate sweet corn and green and wax bean responses to O_3 pollution in southern Ontario by means of detailed observations of the nature and extent of injury during the 1982 and 1983 growing seasons, and by undertaking chemical protectant treatment experiments in the field. The objectives of the study were to determine yield and quality effects, to correlate observed injury with O_3 monitoring data taken at several locations, to evaluate cultivar sensitivity, and to determine the nature of leaf injury and growth retardation.

These objectives were approached by conducting field experiments in 1982 and 1983 at the Cambridge, Harrow and Simcoe Research Stations with evaluation of the response of six cultivars of each species to weekly applications of an O_3 protectant, benomyl. Data on yields and other response variables allowed some assessment of species, cultivar, chemical protectant, and location interactions.

Commercial Importance of Sweet Corn and Green and Wax Beans

Sweet corn is the third most important vegetable crop after tomatoes and potatoes, each of which has about three times the farm value of sweet corn. Approximately 17,000 hectares are grown with a farm value of about $18 million per year. Imports of sweet corn are limited to about 4% of the amount produced within the province. Sweet corn is well-adapted to southern Ontario and is an important contributor to farm income, as well as to the prosperity of the processing industry.

The green and wax bean crop is relatively less important than corn in Ontario accounting for only $5 million cash value to farmers. It could be of greater importance if imports could be replaced by local production. About one third of the green and wax beans consumed in Ontario are imported. Green and wax beans are considered to be well adapted to southern Ontario, yet some factors of the environment may be adversely affecting productivity and profitability. Sensitivity to recurring pollution episodes may be one such limiting factor.

Field Experiments

Replicated field experiments were conducted at the University of Guelph - Ontario Ministry of Agriculture and Food Research Station at Cambridge, Ontario; at the Ontario Ministry of Agriculture and Food Horticultural Experimental Station at Simcoe; and at the Canada Department of Agriculture Research Station at Harrow. At each location, randomized complete block split-plot experiments were conducted with weekly chemical protectant treatments, six cultivars of sweet corn with one planting date, six cultivars of green and wax beans with two planting dates in 1982 and one planting date in 1983, and six blocks in each experiment. The protectant treatments were sprays of water, 1000 ppm benomyl (a commercial fungicide that acts as an antiozonant), and 4000 ppm benomyl on both species in 1982, and sprays of 500 or 2000 ppm benomyl on beans but soil drenches of these concentrations on sweet corn in 1983. The sprays and drenches were applied weekly to all foliage on each plot. The cultivars were

selected from the recommendation lists of the crop committees of the Ontario Ministry of Agriculture and Food.

The experimental design was a split-plot with the six cultivars as whole units and sprays as sub-units. There were three 3-meter long rows in each cultivar whole unit in 1982. In 1983 there were six rows in each whole unit with alternate rows untreated. Spray or drench treatments were sub-units with a different spray treatment on each row in 1982 or different spray or drench treatments on alternate rows in 1983. Row spacing was 90 cm for both species with 4 cm spacing between bean plants and 20 cm spacing between sweet corn plants. Tobacco indicator plants. cultivar 'Bel-W3', were placed on the perimeter of each plot.

Results

Sweet Corn

There was a highly significant cultivar effect at Cambridge with very large differences in yield among cultivars. 'Earlivee' was clearly least productive while 'Golden Jubilee', 'Beacon' and 'Flavorvee' were highest yielding. There was a significant cultivar x benomyl interaction because the total yields of some cultivars were very responsive to benomyl while others were not. The yields of 'Stylepak', 'Golden Jubilee' and 'Earlivee' were enhanced by benomyl. Husk weight, cob number and cob quality (length of cob covered with mature kernels) were highly significantly different among cultivars. Only husk weight and total yield were affected by the interaction. Cob number was significantly affected by benomyl, while quality differed

only among cultivars.

At Harrow, 'Earlivee' was also least productive but 'Beacon' was relatively much lower yielding than at Cambridge. In this case there was an overall significant effect of benomyl with the highest yield at 1000 ppm benomyl and lowest at 4000 ppm. In addition husk weight and quality but not cob number were significantly affected by benomyl.

At Simcoe there were highly significant effects of both cultivar and benomyl, with 'Golden Jubilee' and 'Flavorvee' the most productive. The overall benomyl effect was due to a reduction in yield with 4000 ppm benomyl. Of the three additional response variables, only husk weight was affected by benomyl application.

Green and Wax Beans

There were two plantings of beans, primarily because herbicide injury interfered with plant growth after the first planting at two of the locations. A second planting also allowed assessment of a wider range of O_3 episodes than would have been possible with a single planting, as beans have a shorter seeding to harvest cycle than sweet corn. The benomyl did not significantly affect yield in the first planting at Cambridge while there were highly significant differences among cultivars. 'Contender', 'Bush Blue Lake' and 'Tendergreen' yielded much better than 'Greencrop' or 'Cherokee Wax' while 'Kinghorn Wax' was intermediate. The first planting at Harrow had no significant benomyl effect but the best cultivar was 'Greencrop' with 'Kinghorn Wax' the lowest yielder. The post-planting pre-emergence herbicide was washed deeply into the soil by heavy rains at Simcoe and

severely injured seedlings, especially those of 'Greencrop', 'Bush Blue Lake' and 'Cherokee Wax', with all yields reduced. There was not a significant benomyl effect.

In the second planting at Cambridge most cultivars yielded similarly with only 'Kinghorn Wax' and 'Greencrop' markedly lower yielding. In this case benomyl had a significant overall effect on yield with highest yield with 1000 ppm sprays and lowest with 4000 ppm sprays. At Harrow the array of cultivar differences was somewhat different from Cambridge but 'Kinghorn Wax' was lowest yielding at both locations. There was not a significant benomyl effect. In contrast, the second bean planting at Simcoe had no significant cultivar effect but there was a benomyl effect. The significant benomyl effect seemed to be largely due to an overall decreased yield with 4000 ppm benomyl sprays.

Injury on Ozone-Sensitive Tobacco Plants

Indicator plants of 'Bel W3' tobacco were injured similarly at all three locations. By mid-August all tobacco plants were severely injured up to leaf 10 with injury extending to leaf number 34 to 38. The extensive injury on the plants indicated that substantial doses of O_3 had been received at all locations.

Ozone Concentrations in Southern Ontario in 1982 and 1983

Monitoring data and hourly plots for four Ontario stations were obtained from the Ontario Ministry of the Environment-Air Resources Branch. The Simcoe data were intended to relate to the field experiments at Simcoe. The Kitchener data were for the experiments at

the Cambridge Research Station and the Windsor and Merlin data for the experiments at the Harrow Research Station. The hourly plots revealed that there were a number of potentially injurious O_3 episodes in both years. In 1982 the number of hours with an O_3 concentration greater than 80 ppb was highest at Simcoe followed by Windsor but the ppb-hr dose was about the same at the two locations. The other two locations had much lower durations and doses in 1982. The principal episodes at Simcoe in 1982 occurred in mid-June and mid-July while at Windsor the episodes were in mid-June and early July. The only substantial episode at Kitchener in 1982 was in mid-June.

Ozone concentrations in 1983 were higher in June but not much different in July, August and September, compared with 1982. The plants were in the seedling stage during the June episodes and visible injury symptoms were noted. However, preliminary examination of yield data indicates that the early O_3 injury was not reflected in final yield differences between sprayed and unsprayed plants.

Evaluation of Contaminated Water

and

Soil Sites as Sources

of

Airborne Hazardous Materials

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ABSTRACT

The status of an experimental program supported by the Ontario Ministry of the Environment Air Resources Branch is described in which the aims are to develop methods of quantifying emissions (i.e. source strengths) of organic contaminants from water bodies and from soils. The approach has been to design and operate a small scale system in which water containing dissolved contaminant is subjected to air and water turbulence which can be adjusted to simulate any desired environmental condition, as may exist in a lake, pond, river or lagoon. A similar system has been devised for soils. Attempts are being made to develop relatively simple mathematical models which can be used to correlate the experimental data and predict emission rates in other systems from a knowledge of the water or soil properties and the contaminant's physical chemical properties.

INTRODUCTION.

Although most airborne contaminant sources are "point" in nature, there may be significant emissions to the atmosphere from "non-point" sources such as lakes, rivers or soil. These "non-point" sources may be low in terms of "grams per square metre per second" but since the area is potentially large and the duration long, it is possible that the emissions may represent substantial fractions of the total industrial, municipal, domestic or agricultural emission.

Our aim is to devise two test procedures or protocols which can be used by an environmental agency to predict the likely emission rates by a combination of experiment and calculation based on a sound theoretical basis. The project is planned for three years and at this point in time it is approximately half complete. The water test is nearly complete while the soil test is less advanced.

WATER TEST.

Mass Transfer Coefficient Correlation.

The first stage was a review of the existing literature which was facilitated by a recent EPA sponsored project which we had just completed (Mackay et al. 1982). We also reviewed available test procedures and highlighted some deficiencies in them in a paper co-authored with Smith of SRI (Smith et al. 1983). An important aspect has been the gathering and critical review of data correlating mass transfer coefficients (MTCS) for air-water exchange. A paper describing this aspect was written up and has been published this year in Environmental Science and Technology (Mackay and Yeun 1983). In this work we demonstrated a fundamental difference between laboratory and environmental

MTCs as shown in Figure 1 which is reproduced from that paper. Data have also been gathered for wind speeds over land and water in Ontario in order that these speeds can be inserted into the correlation equations to obtain an estimate of prevailing MTCs under any given wind speed and fetch conditions.

It should be noted that it is accepted that it is necessary to define two mass transfer coefficients; for the air phase (K_G) and the water phase (K_L) and that the reciprocals of these coefficients (i.e. resistances) add in series (with a correction for relative concentrations in the two phases in the form of the Henry's Law Constant (H) or air-water partition coefficient (K_{AW} or H/RT). This represents the Whitman "Two Resistance Approach". In all cases an overall or total resistance or conductivity or MTC is obtained experimentally which may consist of varying proportions of water and air resistance.

EXPERIMENTAL SYSTEMS.

A system was desired to study volatilization rates of organic contaminants that exhibit a wide range of air-water partition coefficients, i.e., Henry's Constant (H). In general, substances with large values of H volatilize rapidly at a rate controlled by the water phase MTC (K_L) and those with low values of H volatilize slowly at a rate controlled by K_G. In this way, the effects of liquid phase and gas phase diffusion control can be examined and applied to the Two Resistance Theory for interphase mass transfer. It is thus desirable to measure the volatilization rates of a series of compounds of varying H in order that from the measured overall MTC the individual values of K_G and K_L can be extracted.
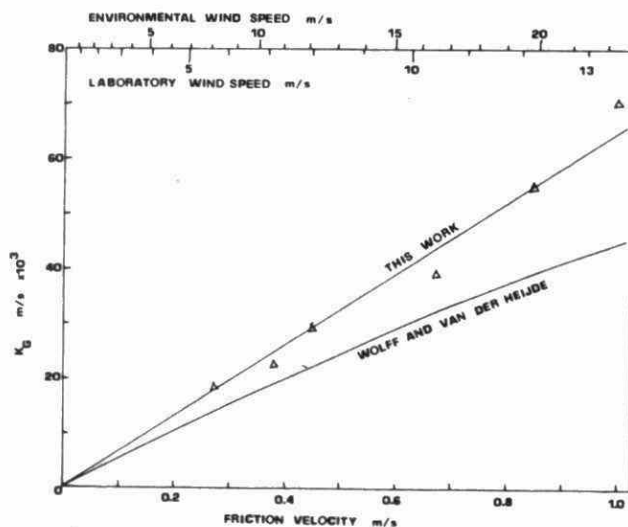
Figure 1a Present K_G correlation (Schmidt number 0.6) and that of Wolff and van der Heijde
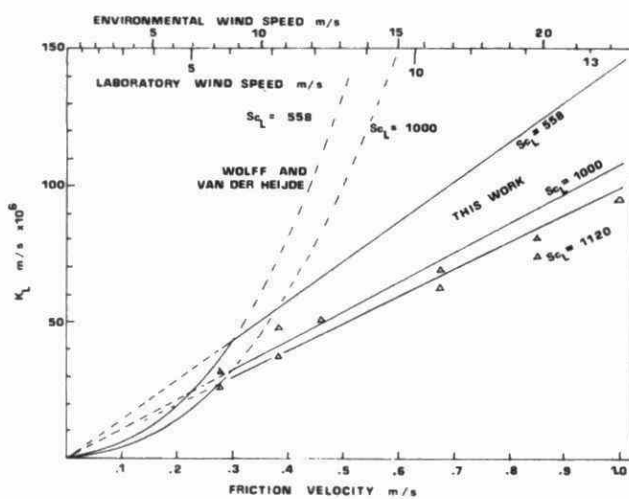
Figure 1b Present K_L correlation and that of Wolff and van der Heijde

An air-water volatilization rate apparatus (Figure 1) has been built and refined. The system is designed to examine the process of volatilization under a variety of conditions. Air phase turbulence can be varied through the use of a blower connected to a rheostat and a flowmeter (calibrated for volumetric air flow rates). Water turbulence can be varied through the use of a propeller powered by a motor and connected to a revolution counter. The system can easily be adapted to study the effects of temperature on the volatilization process as both the air flow and aqueous reservoir can be immersed in temperature-controlled water baths.

Two different analytical methods, gas chromatography (G.C.) and high pressure liquid chromatography (H.P.L.C.) have been used for quantitative analysis. A Hewlett Packard 5840A gas chromatograph and integrator, with a purge and trap sampler Model 7675A is used for the detection of the highly volatile substances. The gas chromatograph is equipped with a flame ionization detector and contains a SE-30 capillary column, with nitrogen as the carrier gas. During the past year, much work has been devoted to perfecting the G.C. systems for the detection of chemicals from the water samples from the volatilization apparatus. The HPLC system is used for the analysis of lower volatility substances such as polyaromatic hydrocarbons and alcohols. The HPLC system consists of an Eldex dual high pressure pump in conjugation with a LDC UV detector (254 nm) and a Hewlett Packard 3390A Integrator. The mobile phase is methyl alcohol: water (80 : 20) and the column is C18 μ Bondapak.

In a typical experiment we measure the decrease in concentration of a mixture of chemicals, present in true solution form, in the aqueous phase.

FIGURE 1
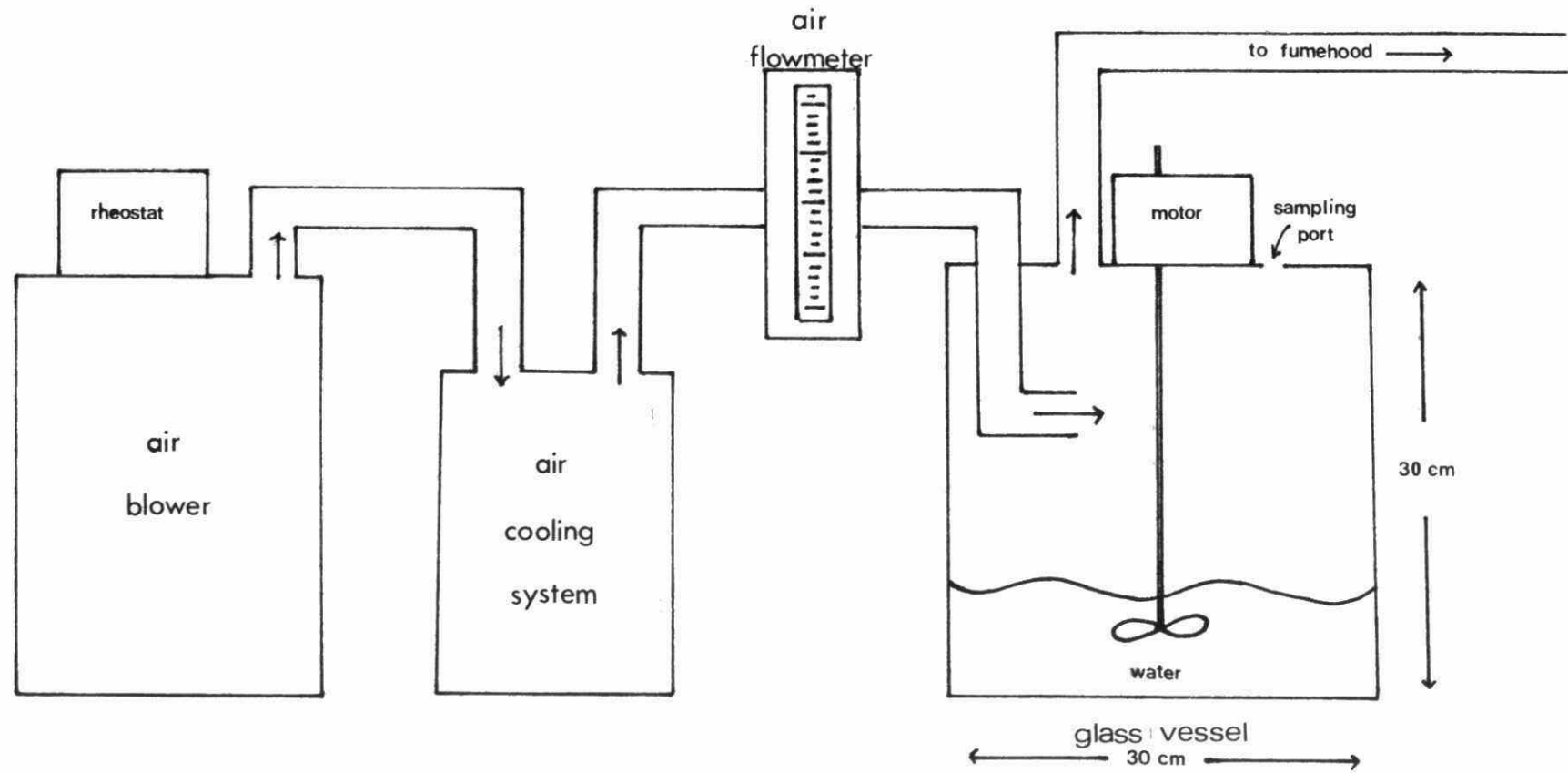
No attempt is made to analyse the air phase. Six litres of distilled water along with an aqueous solution saturated with the substances of interest are added to the glass vessel. Blower speeds and stirrer speeds are set and remain constant during the entire experimental run. During the run, water samples are taken from the glass vessel with a gas-tight syringe and analyzed by either the G.C. or the HPLC system. The concentration decrease with time for each chemical is monitored and a volatilization rate or "overall air-water mass transfer coefficient" is calculated from the slope of the semi-logarithmic plot of concentration versus time.

The time needed for a given experimental run depends on whether rapidly or slowly volatilizing substances are being studied, and ranges from a few hours to several days. In order to explore the effects of liquid phase and gas phase diffusional resistance it is necessary to study a given group of substances under a NxN matrix of air turbulence (i.e blower speeds) and water turbulence (i.e. stirring rates) conditions.

The solutes used have been benzene, toluene, methylcyclopentane, naphthalene, phenanthrene and n pentanol. Unfortunately, there is no convenient hydrocarbon solute (to our knowledge) which has a Henry's Law Constant somewhat smaller than that of phenonthrene. Such a solute would be invaluable in assisting the accurate extraction of K_G values.

RESULTS.

The results have been compiled in the form of overall MTCs from which mean K_G and K_L values have been extracted using the planned variation in H. The K_G and K_L values have been correlated against air

rate (A) and stirrer speed (S) using two equations of the form

$$K_L \text{ or } K_G = K_1 + K_2 A^n S^m$$

where there is a total of 8 constants (i.e. pairs of K_1, K_2, n and m).

A contour plot of K_L versus K_G can then be obtained as shown in Figure 3. On this figure is also plotted the normal range of Ontario lake conditions, as obtained from meteorological data and the K_G and K_L correlations. It is thus possible to adjust conditions in the test system to simulate any desired condition of MTCs and temperature. It is also possible to use natural water (rather than distilled water) to simulate the actual emission conditions.

DISCUSSION.

Present work consists of reprocessing the data to include a diffusivity or Schmidt Number correction factor in the analysis, the investigation of temperature effects, the use of various natural waters and an exploratory study of the effects of surface films. A final test protocol will be written early in 1984. It is expected that the solute of interest will be tested along with three other "bench mark" solutes, probably toluene, naphthalene and phenanthrene. It will then be possible to undertake an experimental investigation of solute emissions from the actual water of concern at the desired temperature and turbulence conditions and obtain experimental data which can be subjected to rigorous theoretical interpretation.

SOIL TEST.

The development of a soil test protocol is more difficult because of the heterogeneous nature of the emitting medium. Two experimental approaches have been taken. The first was the use of a test system
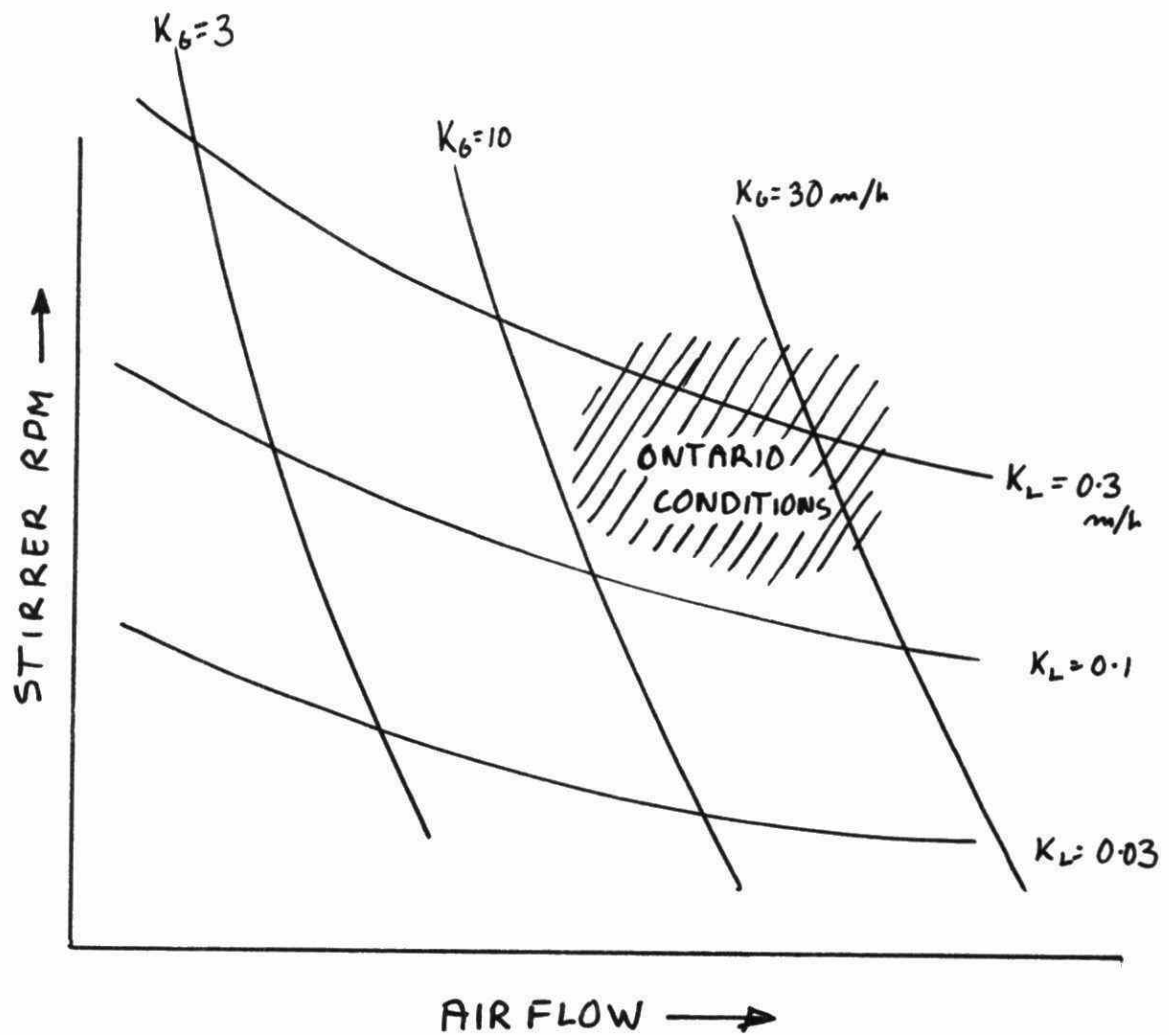
FIGURE 3   ILLUSTRATIVE RESULTS FROM THE
           AIR-WATER TEST SYSTEM

similar to that used for the water test, but containing soil. Although
simple, this system suffers from the difficulty that the soil may not be
in the desired water saturation or sub-saturation condition. It is
recognized that the water "condition" plays an important role in
determining volatilization. Accordingly a more complex system has been
devised and is currently being investigated.

The present apparatus is shown in Figure **4**. In a typical test, the
solute(s) are added to the soil "neat" or in a carrier solution such as
hexane. The volatilized solute is collected and analysed and when the
test is complete the soil column is sectioned into 5 mm layers and
analysed. It is thus possible to obtain a complete mass balance for the
solute. Separately the equilibrium sorption partition coefficient of
the solute is determined. Radio-labelled lindane has been used as the
test solute.

A numerical model has been compiled describing the behaviour of the
solute in the surface soil layer and includes migration into the satu-
rated zone. This model is based on the traditional differential diffu-
sion equation with allowance for partitioning between air, water and
soil, and solute migration in air and water phases. There is little
doubt that this model will be able to describe and correlate the
observed phenomena but it is probably too complex for general use. We
envisage that it may be simplified by rewriting the equations in a
fugacity form, then further simplified to obtain equations which are
more suitable for routine rather than research use.

It is envisaged that the final test protocol will be similar in
principle to that of the water test, but that the experimental procedure

FIGURE 4

APPARATUS FOR
SOIL-AIR EXCHANGE OF
PESTICIDE
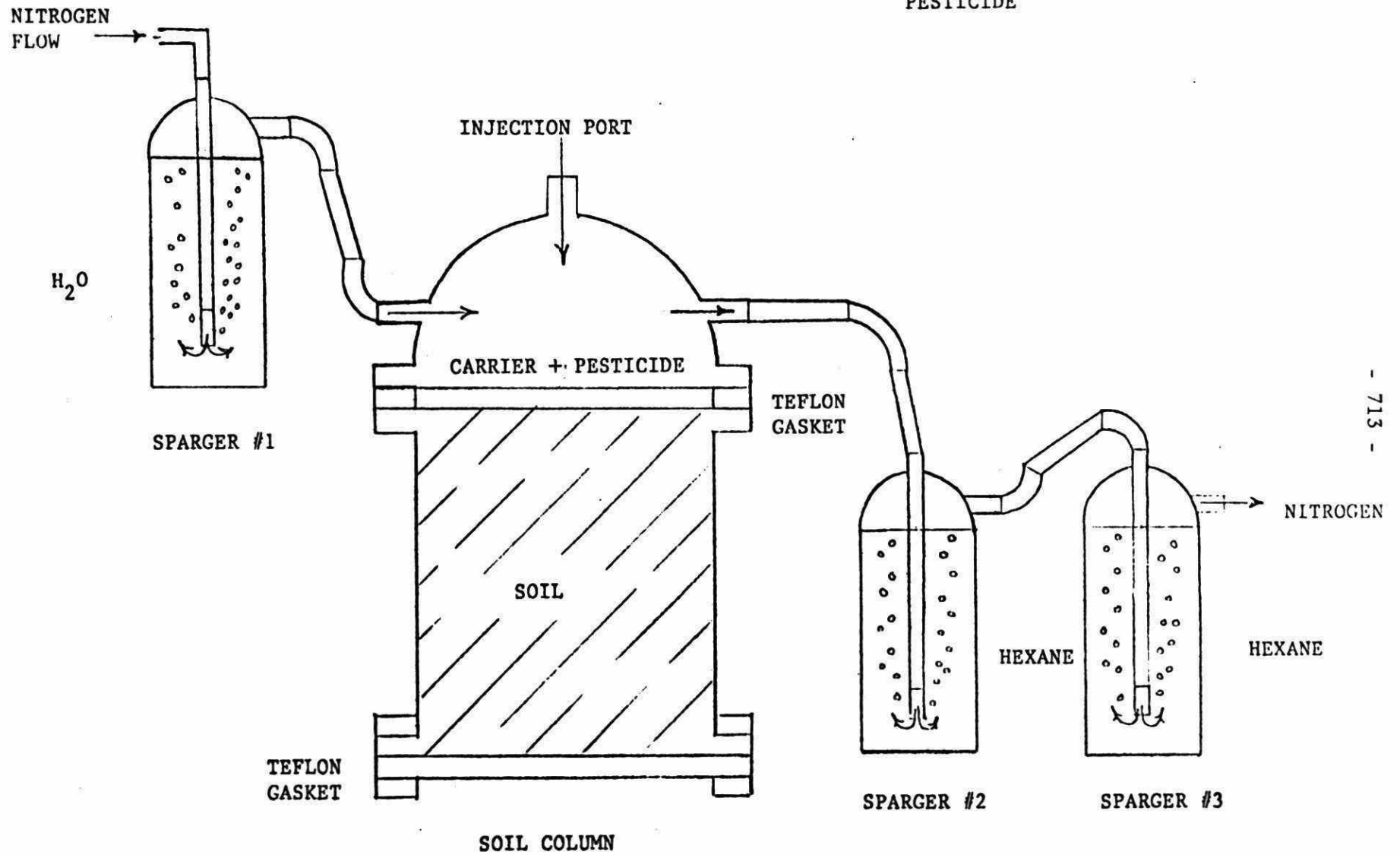
will be more complex. It may consist of taking a sample of the desired soil, adjusting its "water condition", adding the solute of interest with other "bench mark" solutes, determining the diffusion, volatilization characteristics, separately determining the air-water-sorption partitioning properties and finally reconciling the experimental data with a relatively simple mathematical model.

CONCLUSIONS.

The water system is nearing completion with a full description of the recommended procedure expected in early 1984. Most effort is now being devoted to the soil test system in expectation of completion in early 1985.

REFERENCES

Mackay D., Shiu, W. Y., Bobra, A., Billington, J., Chau, E., Yeun, A. T. K., Ng, C. W. K., Szeto, F. "Volatilization of Organic Pollutants from Water" EPA report EPA-600/3-82-019 Washington, D.C. 1982 (NTIS PB 82-230939)

Smith, J. H., Mackay, D., Ng, C. W. K. "Volatilization of Pesticides from Water" Residue Reviews 85 73 (1982)

Mackay, D., Yeun, A. T. K. "Mass Transfer Coefficient Correlations for Volatilization of Organic Solutes from Water" Environ. Sci. Technol 17 211 (1983)

EVALUATION OF ALTERNATIVES TO HIVOL SAMPLING FOR PAH

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INTRODUCTION

One of the most commonly used methods for sampling airborne polynuclear aromatic hydrocarbons (PAH) is the Hi-Vol sampler. This method is fraught with deficiencies because of vapour-phase losses of the more volatile PAH compounds and the reaction of collected material with gases such as ozone, nitrogen dioxide and even nitric acid (van Vaeck et al, 1980; Cautreels and van Cauwenberghe, 1978; Lee et al 1980; Peters and Seifert, 1980; Barton et al, 1978; Brorstrom et al, 1983; Van Vaeck et al, 1979). Most attempts at rectifying these deficiencies have relied on solid sorbents such as Tenax (Cautreels and Van Cauwenberghe, 1978), polyurethane foam (PUF) (Krstulovic et al, 1977; Lindgren et al, 1980; Thrane and Mikalsen, 1981; Yamasaki et al 1982) and XAD-2 Pellizzari E.D., 1979) to capture the volatilized PAH compounds. This approach is, however, still susceptible to the reaction of ozone and other reactive gases with the collected material both on the filter and on the solid sorbent (Jager and Harris 1980; Hughes et al, 1980).

In an effort to develop instrumentation that would mitigate these problems, a research program was undertaken by Concord Scientific Corporation through funding provided by the Ontario Ministry of the Environment, to modify the standard Hi-Vol sampler. These modifications would provide an improved sampling method for PAH while utilizing currently available instrumentation and also allow the collection of large enough samples for chemical and other analyses, such as bioassays.

The modifications to the standard Hi-Vol sampler involved the addition of a backup solid sorbent cartridge and an ozone denuder. The solid sorbents investigated were PUF, XAD-2 and Florisil. Initialy, the use of C_{18} GC Bondapak on Porasil was considered, but its high cost and the reported high pressure drop across this material (Lindgren et al) rendered it unsuitable for routine use in a network of modified Hi-Vol samplers.

The provision of an ozone denuder preceeding the Hi-Vol filter required the complete design, development and fabrication of this device. The selective removal of ozone from ambient air during sampling has not been reported previously. It was therefore necessary to find a suitable material that would act as a near perfect sink for ozone yet not remove any vapour phase PAH from the sample air stream.

Laboratory and field tests are described in which candidate solid sorbents (PUF, XAD-2 and Florisil) were investigated and laboratory and field ozone denuders were developed and tested. Field evaluation of the modified Hi-Vol samplers is also described.

EXPERIMENTAL

Construction of samplers: Laboratory Scale Sampler

Laboratory scale samplers consisted of a glass fibre filter (47 mm diameter) contained in a polyethylene filter holder and a series of 1 to 3 custom fabricated stainless steel cartridges. The cartridges were fabricated to allow separate amounts of the sorbents to be used. See Figure 1. The sample flow rate through the laboratory scale samplers was such that the face velocity was similar to that in a Hi-Vol sampler operating at a nominal 20 CFM flowrate.

Construction of modified Hi-Vol samplers

The standard Hi-Vol sampler was modified by placing a cartridge between the filter support (plenum) and the motor. Figure 2 shows the cartridge system used. This system allows great flexibility in the amounts of sorbent that may be employed and was accomplished by the use of variable lengths of a polyethylene insert and as many screens (to separate each sorbent bed) as desired.

47 mm ϕ polyethylene filter holder

adapter fittings

upper section

Aluminum re-enforced SS screen

20 mm section

40 mm section

50 mm section

Lower section
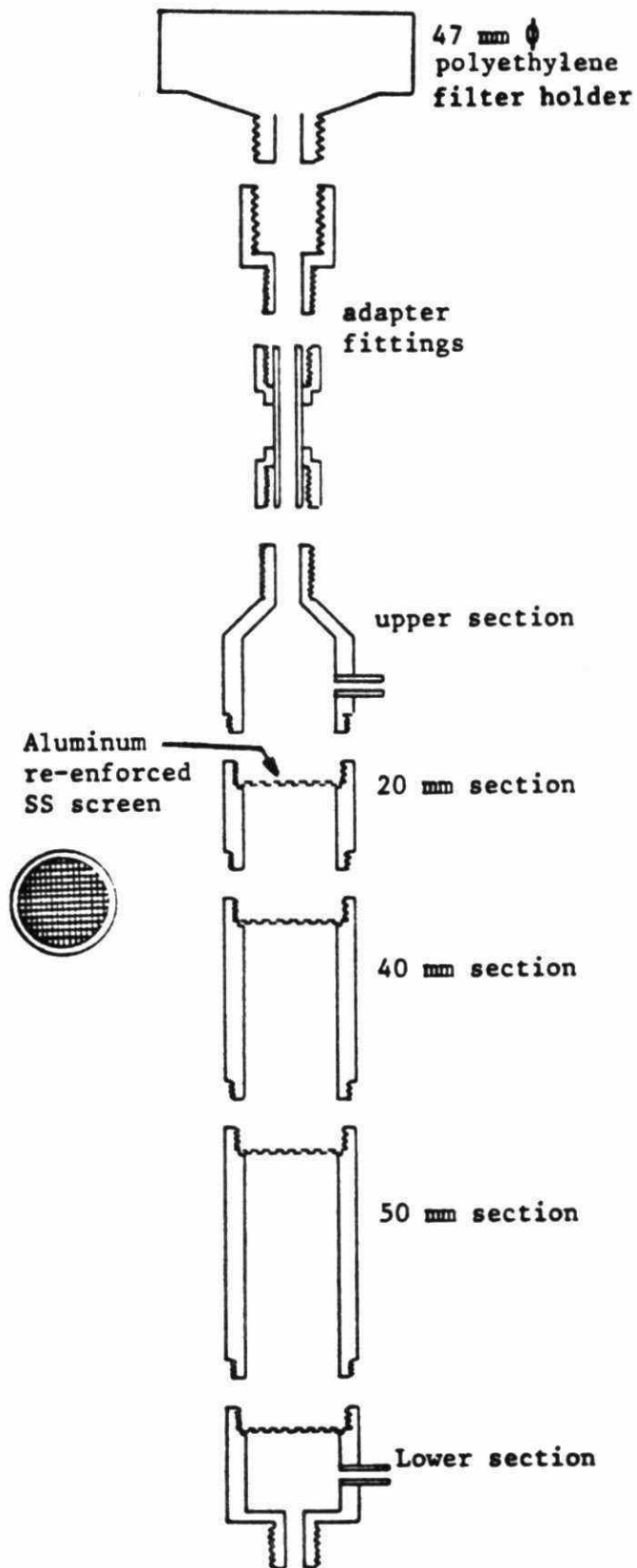
Figure 1   Schematic of Laboratory Test Chamber Filter/Sorbent Cartridge

Transport Cap (Lower cap not shown)

fits to
plenum of
HiVol Filter

Polyethylene insert
and SS screen
"Sandwich"
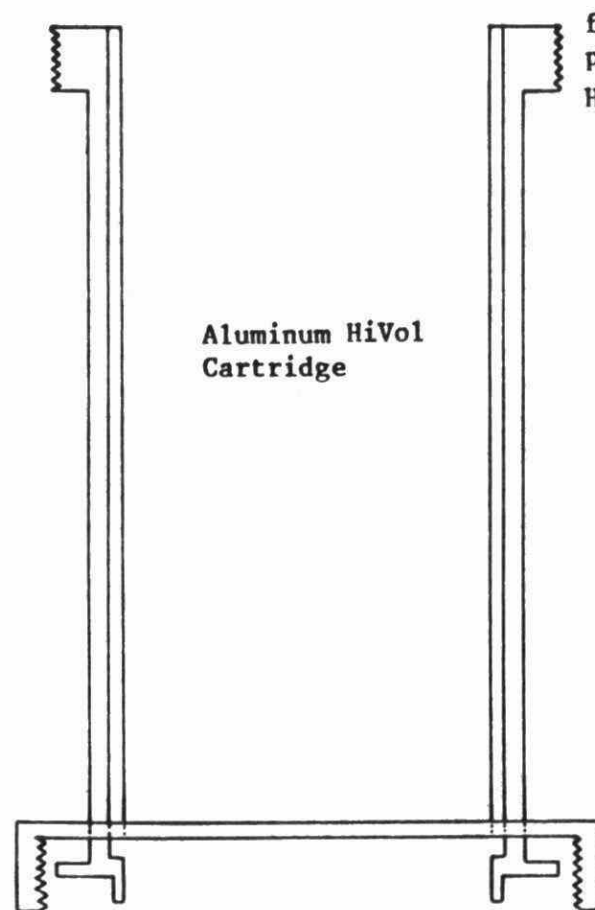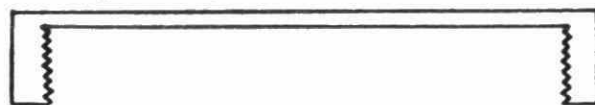
Perforated Plate
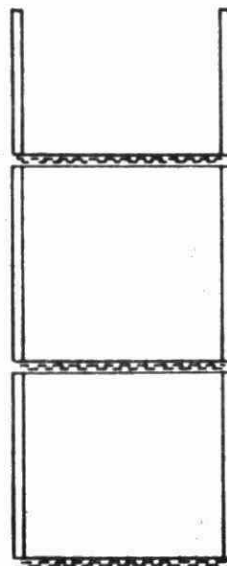
Aluminum HiVol
Cartridge
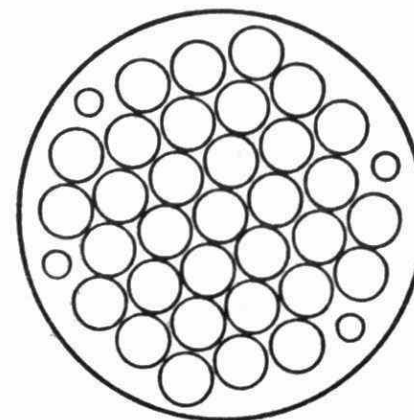
Collar: Fits
onto HiVol
Motor

Figure 2   Details of HiVol Sorbent Cartridge

The field scale denuder-Hi-Vol-cartridge system developed is illustrated in Figure 3.

The standard Hi-Vol housing was extended and covered with the standard Hi-Vol cover to protect the denuder bundle and filter from the elements. The denuder bundle consisted of over 1250 Kraft paper tubes, each 61 cm long and 7.9 mm ID. The intersticial channels in the bundle were sealed so that all the air flow was through the tubes. The denuder bundle was seated in a support tray and the seating was rendered air-tight to ensure that all the airflow through the filter and cartridge was directed through the denuder tubes. Measurements of the air velocity 1 cm above the bundle were made while sampling at 15 cfm. Measurements taken at 16 locations were in a 5% range, indicating even flow through the complete bundle.

Field Sampling and Laboratory Experiments

Field sampling was conducted on the roof of the Hamilton Beach Rescue Unit Association (HBRUA) building on Beach Boulevard in Hamilton. Sites in Hamilton have been shown to have significant ambient PAH concentrations as a result of nearby coke oven emissions (Katz et al. 1980). The details of the location of the sampling site relative to the MOE ambient monitoring station and the Stelco and Dofasco facilities are shown in Figure 4.

A

DENUDER
BUNDLE
(61 cm bundle
length)

SUPPORT TRAY

EXTENSION HOUSING

STANDARD
HiVOL SHELTER

PLENUM

TO HiVOL
MOTOR

HiVOL
FILTER

DENUDER BUNDLE

FILTER CASSETTE

VIEW LOOKING DOWN AT
POINT "A"
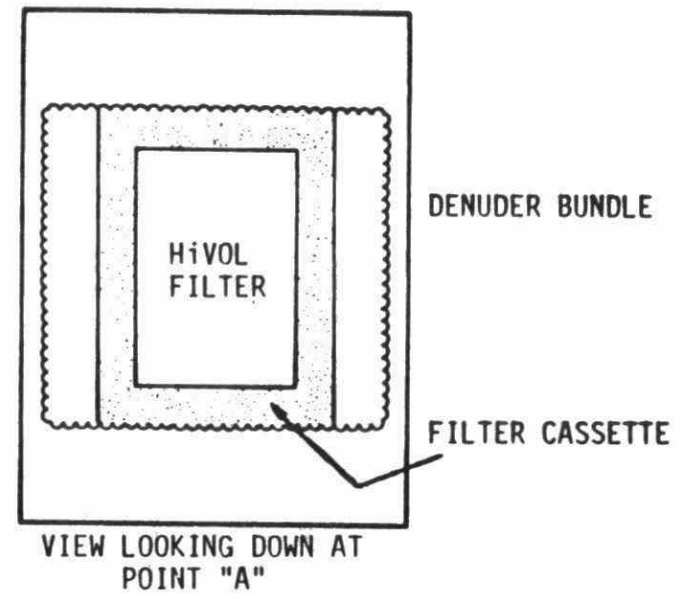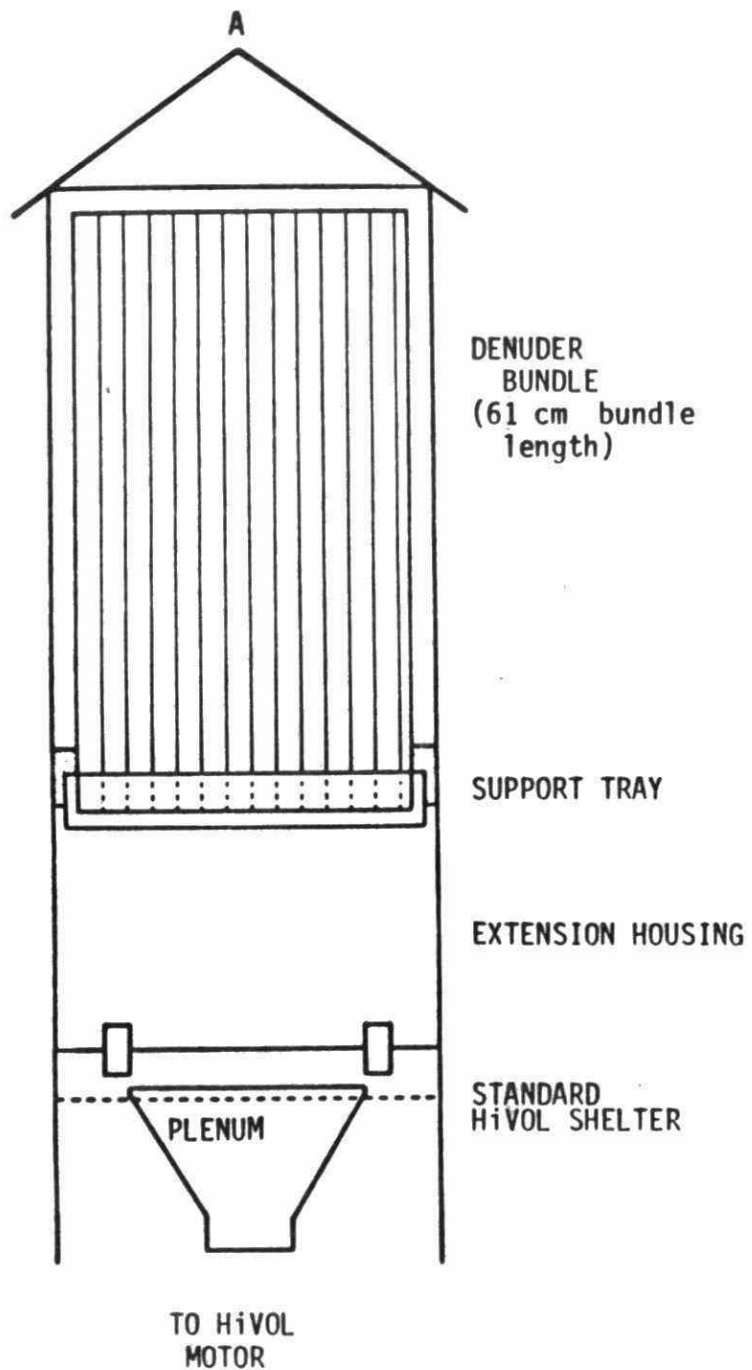
Figure 3   SCHEMATIC OF HiVOL OZONE DEUNDER
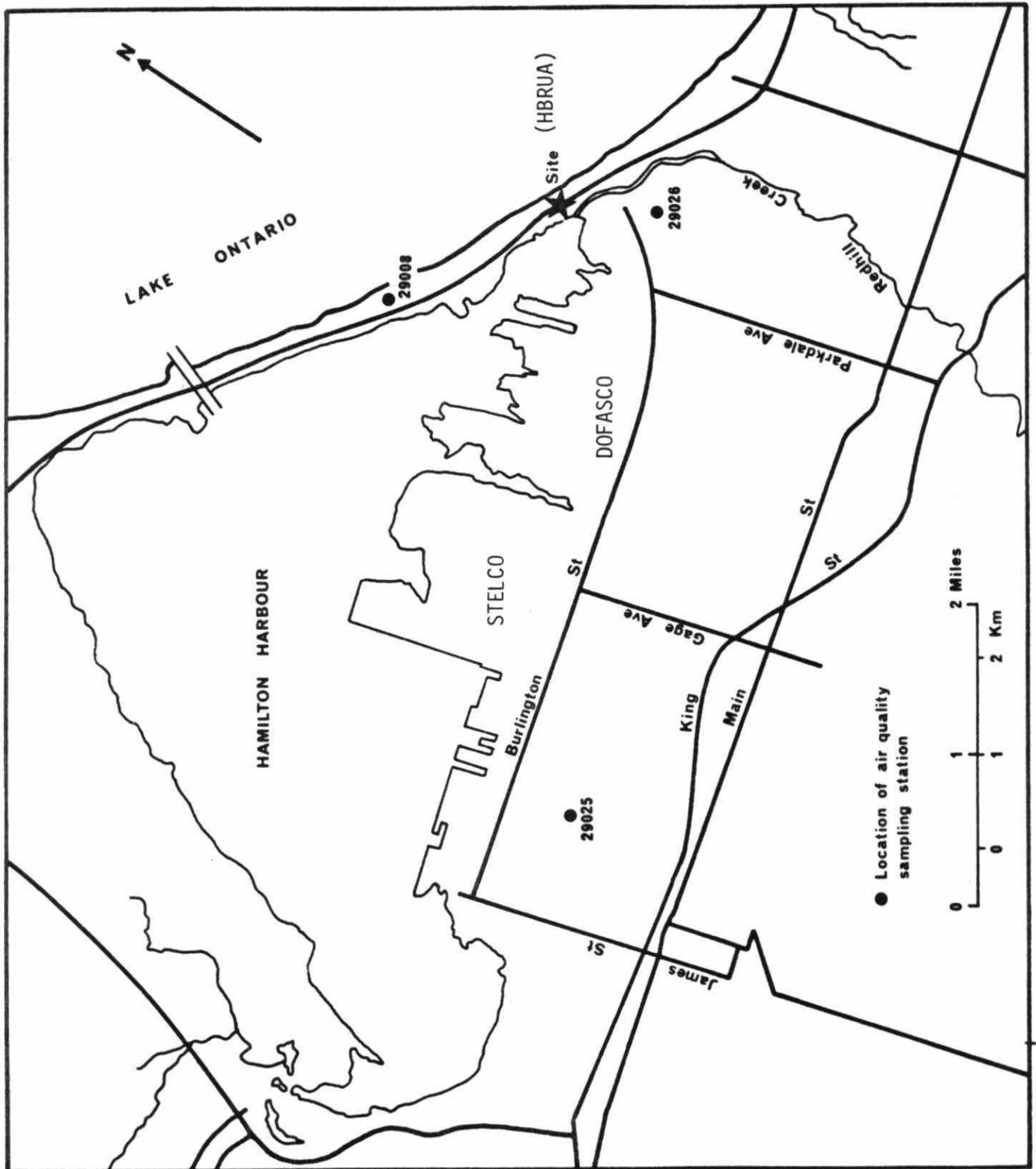
Figure 4

Location of Sampling Site in Relation to Existing
MOE Sites and Major Pollution Sources

Sampling was performed only on days on which the forecasted wind direction was favourable (SSW) and in the case of summertime sampling, when ambient ozone levels were anticipated to be high.

Field sampling comprised the following:

a) Standard Hi-Vol sampling for the collection of filters for laboratory experiments

b) Determination of the pressure drop across the various filter/cartridge combinations

c) Sample collection for comparing the retention of PAH on filters and sorbents in the standard and modified Hi-Vols

d) Comparison of the mass loadings between the standard and modified Hi-Vol samplers

Laboratory experiments were conducted to determine the following:

a) the relative pressure drop versus flow rate relationships for the sorbents

b) the relative efficiency of the backup sorbents

c) the effect of ozone concentration on PAH losses

d) the effect of using the ozone denuder for PAH sampling

For these laboratory runs an air stream was drawn through PAH-laden filters. The volatilized PAH compounds were collected on backup sorbent plugs or a cryogenic trap. The PAH-laden filters were cut (as required) from Hi-Vol filters which were exposed for sample collection at the site near Hamilton.

All samples were stored in a freezer at -15 °C until analysed. Filters and sorbents were analysed for a minimum of 6 PAH compounds (selected to include carcinogenic or mutagenic PAH compounds with a range of ring structure and hence volatility) by HPLC coupled with fluorescence detection. Samples and blanks were extracted in cyclohexane (soxhlet extraction) followed by concentration to near dryness under reduced pressure and resolubilization in methanol.

The HPLC chromatograms were obtained using a Waters ALC/GPC 244C liquid chromatograph equipped with a 254/375 nm fluorescence detector. Excitation was at 254 nm with fluorescence detection using a 375 nm filter. A Radial Pak 5 μm column with a methanol/water (75:25 v/v) eluting solvent grading to 100 % methanol was used. At a later stage a Vydac C18 201TP54 column using a 50:50 acetonitrile: water eluting solvent grading to 100 % water was used.

RESULTS AND DISCUSSION

The relationships between the pressure drop across a 2 cm bed depth of sorbent and the flow rate were examined for the three sorbents-PUF, XAD-2 and Florisil using the laboratory scale sampler. (See Figure 5). As expected, PUF has the lowest pressure drop and florisil the highest. These tests indicated what practical amounts of sorbents could be used in Hi-Vol samplers. It should be recognized that the life of Hi-Vol motors is highly dependent on the pressure drop across the glass fibre filter/sorbent cartridge combination.

Each of the modified Hi-Vols contained two or three sections of the same sorbent. For these modified Hi-Vol samplers, the typical relationships between the measured pressure drop (indicated as the vacuum in inches of water) for various sorbents and the flow rate are indicated in Table 1. A flow rate of 15-20 cfm was the target and this was easily achieved with ~ 2.5 cm bed depths of XAD-2 and PUF or 2 cm of florisil.

The relative performance of the three sorbents was evaluated given the requirement for a sampling rate of 15 - 20 cfm. Three separate sections of PUF and XAD-2 and two sections for florisil backup sorbents were employed in field runs. Analytical data are presented in Table 2 in which the flow rates and the percentages of each of six PAH compounds retained by the filter and each sorbent section are tabulated.
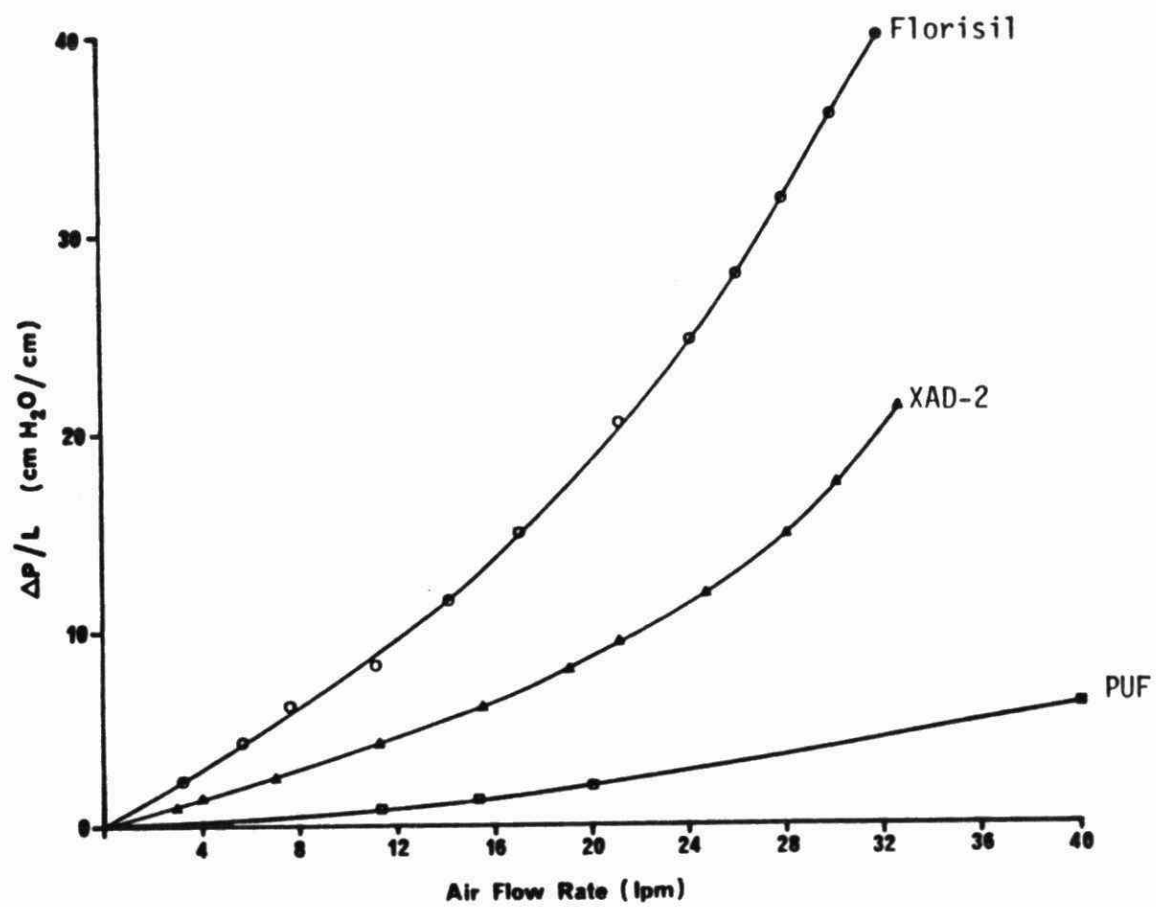
<u>Figure 5</u>   Relationship Between Pressure Drop and Flow Rate for Sorbents

Table 1

Pressure Drop in Various Hi-Vol
Filter/Sorbent Cartridge Samplers

Test Item	Measured Vacuum " H_2O	Measured Flow Rate (CFM)
Filter, empty cartridge	20	63-65
Filter, cartridge with		
2.54 cm depth foam (4.06 g)	43	35-37
5.08 cm depth (8.1 g)	>50	21-23
11.2 cm depth (16.2 g)	>50	13-15
Filter, cartridge with		
2.54 cm depth XAD 2 (39.4 g)	>50	24-26
Filter, cartridge with		
2.54 cm depth florisil (58.6 g)	>50	10-12
Filter, cartridge with		
2.54 cm depth foam (4.06 g)	20	20**
Filter, cartridge with		
44 g XAD (~2.8 cm bed)	43.5	20**
Filter, cartridge with		
38 g florisil (~1.6 cm bed)	40	20**

** Flow set to 20 CFM.

Table 2

Relative Performance of Various Filter/Sorbent Cartridge Systems

Sampler	S #	Sample	% of Total Analysed					
			F	P	B(a)P	3 MC	B(ghi)P	1 2 4 5-D
Std. H-Vol	41	Filter	100 (4.5)*	100 (4.6)	100 (3.2)	100 (0.8)	100 (1.6)	100 (2.7)
PUF	8	Filter	21	11	91	87	< 64	86
		Section 1	78	87	4	< 6	< 25	8
		Section 2	0.5	0.3	< 0.3	< 2	< 6	3
		Section 3	1 (59)	1 (80)	5 (8.1)	5 (1.0)	< 6 (5.8)	3 (5.1)
XAD-2	14.5	Filter	11	11	54	55	54	64
		Section 1	82	81	35	29	40	28
		Section 2	5	5	7	8	3	4
		Section 3	2 (35)	4 (26)	4 (4.6)	9 (0.6)	3 (5.9)	4 (1.9)
Florisil	16	Filter	23	30	99.1	94	77	92
		Section 1	62	41	< 1	< 5	21	7
		Section 2	15 (35)	29 (24)	0.1 (5.6)	< 0.6 (0.8)	2 (3.2)	1 (3.5)

S#   = Sampling rate in cfm
F   = Fluoranthene   P   = Pyrene
B(a)P   = Benzo(a)pyrene   3 MC   = 3-Methylcholanthrene
B(ghi)P = Benzo(ghi)perylene   1245D = 1,2,4,5 - Dibenzopyrene
* Numbers in parentheses are total concentration in ng/m^3

The data in Table 2 indicate that most losses from the filter are incurred by fluoranthene (F) and pyrene (P). PUF appears to be most effective in capturing PAH compounds but this may be due to a lower sampling rate for the particular test. For the less volatile PAH compounds, the standard Hi-Vol yielded PAH concentrations similar to those obtained using the filter/sorbent cartridge system.

A modified Hi-Vol (with 2 sections of XAD-2 back up sorbent) and an Andersen cascade impactor (Hi-Vol with Andersen head) were compared in side-by-side sampling. The PAH collected on the filters are given in Table 3. The filter in modified Hi-Vol collected about 50% more of each PAH than the Andersen filters. All filters in the Anderson sampler were combined for the analysis. It should be noted however that the sampling rate for the Andersen sampler is nearly three times larger than that for the modified Hi-Vol. Analogous comparative experiments by Katz and Chan (Katz & Chan, 1980) indicated that the Andersen sampler collected nearly twice as much PAH than the standard Hi-Vol. However in those experiments, the Andersen sampler was operated at the lower flowrate (by a factor of 2.5). It is likely that the sampling rate will be dominant in determining the losses of PAH from filters.

The effects of ozone on the retention of PAH compounds on the filter and sorbents and the efficacy of the use of the ozone denuder were studied under laboratory and field conditions. The PAH losses from PAH-laden filters through which air with or without ozone was passed are given in Table 4. For this laboratory study, all the PAH-laden filter discs used (47 mm diameter) were obtained from a single Hi-Vol filter. The second column in Table 4 gives the mean and standard deviation of the weight of each PAH on three other discs (47 mm diameter) cut from the same Hi-Vol filter as that used for the loss experiments.

The data in the third and fifth columns, (labelled U) in Table 4 are the weights of PAH on discs with the same history (except for passage of air through them) as the discs used in columns labelled E_1, E_{2A} and E_{2B}.

In the absence of ozone (columns U and E_1), there were the expected losses of flouranthene and pyrene similar to those indicated in Table 2. These losses were recovered on backup florisil sorbent cartridge (first section). For the heavier PAH compounds, there were no apparent losses from the filters (back up sorbent cartridges showed no detectable quantities of these PAH compounds).

Table 3

Collection of Selected PAH on Filters from

Modified HiVol and Andersen Cascade Impactor

PAH	Concentration (ng/m^3)		Ratio
	HVX*	HVA#	HVX/HVA
F	13.6	8.5	1.6
P	11.3	7.5	1.5
B(a)P	5.9	4.7	1.3
3MC	1.9	1.3	1.5
1245-D	2.6	2.0	1.3
B(b)F	11.6	8.0	1.5
B(k)F	6.4	4.4	1.5

* HiVol with XAD-2 cartridge. 570 m^3 sample volume. Flow rate 14 cfm

HiVol with Anderson Head. 1620 m^3 sample volume. Flow rate 40 cfm

F	= Fluorene	P	= Pyrene
B(a)P	= Benzo(a)pyrene	3MC	= 3 Methylcholanthrene
1245D	= 1,2,4,5 Dibenzopyrene	B(b)F	= Benzo(b)fluoranthene
B(k)F	= Benzo(k)fluoranthene		

Table 4

Effects of Ozone on Retention of PAH Compounds in
Laboratory Filter/Sorbent Cartridge Sampling Systems

PAH	Weight in ng on Filter					
	Mean ± SD	U	E_1	U	E_{2A}	E_{2B}
F	162 ± 34	142	50	108	54	58
P	126 ± 31	54	32	87	15	52
B(a)P	103 ± 25	112	123	119	48	47
3 MC	48 ± 10	25	30	27	15	14
B(ghi)P	<36 ± 14	9	6	ND	ND	ND
1 2 4 5-D	65 ± 17	85	97	65	32	33

U   Unexposed filter
E_1 Exposed filter, $[O_3]$ = 0
E_{2A} Exposed filter, $[O_3]$ = 190 ppb
E_{2B} Exposed filter, $[O_3]$ = 190 ppb
ND   Not Detected

F	= Fluoranthene	P	= Pyrene
B(a)P	= Benzo(a)pyrene	3 MC	= 3-Methylcholanthrene
B(ghi)P	= Benzo(ghi)perylene	1245D	= 1,2,4,5 - Dibenzopyrene

For the runs in which ozone was present (columns E_{2A}, E_{2B}), there were losses of the heavier PAH compounds from the filter and none of these compounds were detected in the backup sorbents. In the case of fluoranthene and pyrene, the losses are similar to those in the absence of ozone but the back-up sorbent (florisil) collected substantially more fluoranthene (especially) and pyrene than was expected to be lost from the filter. It is suspected that an interfering compound in the HPLC analysis may account for this observation.

Ozone Denuder Material Evaluation

Several materials were evaluated as candidates for use in the ozone denuder. Screening tests were conducted using inlet ozone concentrations of up to 450 ppb at temperatures ranging from 0 to 30 °C. Two of the most promising materials - latex and Kraft tubing -were selected for more exhaustive and stringent testing. Table 5 summarizes the removal efficiencies obtained under varying conditions of temperature, duration of testing and $[O_3]$. Paper-based tubing was found to be superior, however kraft tubing was mechanically stronger and more readily available, and was therefore utilized on the construction of the full-scale denuder.

Table 5

Typical Results of Removal Efficiency of

Candidate O_3 Denuder Materials

Tube Material	Temp.	Duration	$[O_3]$	Removal Eff.
	°C	hrs	ppb	%
Kraft	22	24	166	70
	-15°C	24	127	52
Paper	22	25	117	74
	-15	2	117	62
Latex	22	24	133	83
	-15	1	157	7
Latex-MnO_2	22	24	126	95 - 100
	-15	3	125	13

Results from a laboratory experiment in which a small scale ozone denuder was employed in front of the PAH laden filter discs at the same time that another disc was exposed to ozone, are given in Table 6. The columns U, E and D refer respectively to filter discs which were unexposed, exposed to 190 ppb O_3, and exposed with a denuder in place.

The data clearly show that the denuder is effective in reducing losses of PAH compounds.

Field runs in which the modified Hi-Vols with and without the ozone denuder in place were conducted to determine the effectiveness of the ozone denuder. These runs were performed in the late summer on days when ambient temperatures and ozone concentrations were relatively high. During the sampling period, the mean ozone concentrations at the nearest MOE monitoring station ranged from 20 to 55 ppb and the mean temperatures ranged from 22 to 29 °C. Analytical data from these experiments are not yet available.

Measurements of ozone concentrations at the inlet to the ozone denuder and in the space between the outlet of the denuder and the filter paper indicated a removal efficiency of ~ 80%. Ambient concentrations were ~ 30 ppb. Similar measurements in which the ambient air stream was spiked with ozone from an ozone generator, gave removal efficiencies of 63 - 71% at inlet ozone concentrations of 30 to 70 ppb. The

Table 6

Laboratory Experiments to Investigate Effectiveness

of Ozone Denuder in Reducing PAH losses from Filters

PAH	Weight in ng on Filter				
	Mean ± SD	U	E	E	D
F	162 ± 34	99	17	22	41
P	126 ± 31	133	35	39	78
B(a)P	103 ± 25	108	14	18	54
3 MC	48 ± 10	33	9	12	27
B(ghi)P	36 ± 14	ND	ND	ND	ND
1245-D	65 ± 17	53	8	9	26

U Unexposed filter

E Exposed filter $[O_3]$ = 190 ppb

E Exposed filter $[O_3]$ = 190 ppb

D Exposed filter $[O_3]$ = 190 ppb but preceded by denuder

F   = Fluoranthene   P   = Pyrene

B(a)P   = Benzo(a)pyrene   3 MC   = 3-Methylcholanthrene

B(ghi)P = Benzo(ghi)perylene   1245D = 1,2,4,5 - Dibenzopyrene

maintenance of constant ozone concentration at the inlet of the ozone denuder was difficult under field conditions. Analagous laboratory experiements conducted in an 8 m^3 chamber in which stable ozone concentrations between 100 and 140 ppb were established, showed that the removal efficiency of the Hi-Vol ozone denuder was better than 98%.

SUMMARY AND CONCLUSIONS

The standard Hi-Vol sampler can easily be modified by the addition of a versatile sorbent cartridge and if required an ozone denuder. PUF was found to be the most appropriate sorbent (compared to XAD-2 and Florisil) in view of its greater efficiency in trapping volatilized PAH compounds and also due to its lower resistance to flow. The use of PUF will allow adequate flow rates (15-20) cfm) without significantly reducing the life of Hi-Vol motors.

Laboratory and field experiments with an ozone denuder utilizing kraft paper as the sink for ozone demonstrated the efficency of the denuder in reducing the ozone concentration to which the PAH compounds (on the filter and on the sorbent) are exposed. The addition of an ozone denuder and backup sorbent cartridge to a Hi-Vol sampler have been demonstrated to be easily accomplished. The costs for these modifications are not likely to be excessive.

These modifications should significantly improve the methodology for ambient PAH sampling. The extension of the application of the denuder technology to the removal of NO_2 from the air stream will represent the next major contribution to the advancement of the sampling methodology for PAH. These advancements are extremely important in view of the reported higher mutagencity of some nitro PAH compounds (Schuetzle, 1983). It is therefore esential to establish whether the nitro- as well as oxygenated PAH species do occur in the atmosphere or whether they are artifacts resulting from the reactions of NO_2 and O_3 with material collected on the filters.

ACKNOWLEDGEMENT

All HPLC analyses were carried out by Mr. Steve Jenkins of IEC-Beak Analytical Services, Toronto.

REFERENCES

Adams J., Menzies K. and Levins P. (1977). Selection and evaluation of sorbent resin for the collection of organic compounds. U.S. EPA-600/7-77-044. EPA Office of Research and Development, Industrial Evironmental Research Laboratory Research, Triangle Park N.C. 27711.

Barton S.C., Johnson N.D., Das B.S. and Caton R.B. (1980). PAH losses during high-volume sampling. Presented at APCA International Technical Conference on Toxic Contaminants, Niagara Falls, New York, 1980.

Brorstrom E., Grennfelt, P., Lindskog, A. (1983). The effect of nitrogen dioxide and ozone on the decomposition of particle associated polycyclic aromatic hydrocarbons during sampling from the atmosphere. Atmos. Environ. 17, 601.

Cautreels, W., van Cauwenberghe, K. (1978). Experiments on the distribution of organic pollutants between airborne particulate matter and the corresponding gas phase. Atmos. Environ. 12, 1133.

Hanson R.L., Clark C.R., Carpenter R.L. and Hobbs C.H. (1981). Evaluation of Texax-GC and XAD-2 as polymer adsorbents for sampling fossil fuel combustion products containing nitrogen oxides. Env. Sci. Technol. 15, 701.

Hughes, M.M., Natusch, D.F.S., Taylor, D.R. and Zeller, M.V. (1980). Chemical transformations of particulated polycyclic organic matter. In Polycuclear Aromatic Hydrocarbons: Chemistry and Biological Effects (Edited by Bjorseth, A. and Dennis, A.), Battelle Press, Columbus, Ohio.

Jager, J., Hanus, V. (1980). Reaction of solid carrier or desorbed polycyclic aromatic hydrocarbons with gaseous low-concentration nitrogen dioxide. J. Hyg. Epidem. Microbiol. Immun. 23,1.

Katz, M. and Chan C. (1980). Comparative distribution of eight polycyclic aromatic hydrocarbons in airborne particulates collected by conventional high-volume sampling and by size fractionating. Env. Sci. Technol. 14, 838.

Krstulovic, A.M., Rosie D.M. and Brown P.R. (1977). Distribution of some atmospheric polynuclear aromatic hydrocarbons. Int. Lab. September/October, 11-18.

Lee, F. S-C., Pierson, W.R., Ezike, J. (1980). The problem of PAH degradation during filter collection of airborne particulates - An evaluation of several commonly used filter media. In polynuclear Aromatic Hydrocarbons. Chemistry and Biological Effects. (Edited by Bjorseth, A. and Dennis, A.J.) pp. 543-563. Battelle, Press. Columbus, Ohio.

Lindgren, J.L., Krauss H.J. and Fox M.A. (1980). A comparison of two techniques for the collection and analysis of polynuclear aromatic compounds in ambient air. J. Air Poll. Contr. Assn. 30, 166.

Pellizzari, E.D. (1979). Ambient air carcinogenic vapours: Improved sampling and analytical techniques and field studies. U.S. EPA-600/2-79-081. Enviornmental Sciences Research Laboratory Research Triangle Park N.C. 27711.

Peters, J. and Seifert B. (1980). Losses of benzo(a)pyrene under conditions of high volume sampling. Atmospheric Environment 14, 117-119.

Rajagopalan, R., Vohra, K.G., Rao, M. (1983) Studies on oxidation of benzo(a)pyrene by sunlight and ozone. Science of the Total Environment 27, 33.

Schuetzle, D. (1983) Biological tests in the evaluation of mutagenicity and carcinogenicity of air pollutants. Environ. Health Perspectives J. 47, 65.

Thrane, K.E. and Mikalson A. (1981). High-volume sampling of airborne polycyclic aromatic hydrocarbons using glass fibre filters and polyurethane foam. Atmos. Environ. 15, 909.

van Vaeck, L., Brodden, G., van Cauwenberghe, K., (1980). On the relevance of air pollution measurements of aliphatic and polyaromatic hydrocarbons in ambient particulate matter. Biomed. Mass Spectrometry 7, 473.

van Vaeck, L., Brodden, G., Cautreels, W., van Cauwenberghe, K., (1979). Aerosol collection by cascade impaction and filtration: Influence of different sampling systems on the measured pollutant levels. Science of the Total Environment 11, 41.

Yamasaki H., Kurvata K. and Miyamoto (1982). Effects of ambient temperature on aspects of airborne polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 16, 189.

Laser Induced Emission Spectroscopy of Polycyclic Aromatic Hydrocarbons
(PAH) in Low Temperature Matrices

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ABSTRACT:

Accurate identification and determination of trace amounts of
PAH compounds in environmental samples is a prerequisite to any
sensible attempt to set clean air standards. Emission and absorption
spectroscopy have provided satisfactory data for inorganic compounds,
but have been hampered from doing so for PAH compounds by the broad
spectral features of such compounds at room temperature. Moreover,
characteristic spectral lines are needed for a useful identification and
determination technique. Shopolskii spectroscopy, in which samples are
dissolved in normal albeanes (e.g. heptane) and then cooled to low
temperatures (15°K), can give the necessary narrow line-like features.
Then, radiation of any wavelength shorter than the threshold for a
transition from the ground state of the PAH compound to its first
singlet electronic level can be used to produce emission characteristic
of that compound. However, some excitation wavelengths will be more
effective than others, and these wavelengths will vary from compound to
compound. Hence a tunable, high intensity, narrow band excitation
source is desirable. We use a pulsed, tunable dye laser in conjunction
with a closed cycle helium refrigerator to investigate Shpolskii spectra
of individual PAH compounds. Then, these spectra can be used to
identify and determine particular PAH compounds present in environmental
samples. We shall describe our technique, discuss our results for
some PAH compounds, and also describe our initial attempts to analyse
environmental samples obtained in the Hamilton, Ontario area by
Concord Scientific Corportation of Toronto.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) occur as trace pollutants in the atmosphere, in soil, and in water.[1] Some of them are known carcinogens e.g. benzo(a)pyrene or mutagens [e.g. dibenz (a,h) anthracene]. Their derivatives and heterocyclic relatives [e.g. 1-nitrobenzo(a) pyrene] can be even more potent as mutagens, and possible carcinogens (2). To set sensible air quality standards, we need to know what levels of these PAH compounds can be tolerated, and what levels exist in the local environment. The former need is medical and biological in character, and is outside our expertise. It is the latter problem, largely analytical in nature, which has interested us.

PAH compounds arise from combustion process, and from atmospheric chemical reactions. The same is true of their derivatives and related heterocyclic compounds. They, along with derivative and related compounds, number in the thousands. This huge number presents considerable analytic difficulty in their identification and quantification. Chromatography in its various forms and in conjunction with fluorescence spectrometry and/or mass spectrometry has been a standard tool for analysis. Continual refinements to the basic chromatographic techniques have been required to keep abrest of ever increasing problems (2). Nonetheless, the resolution of chromatographic techniques is limited, and the number of PAH compounds is enormous. One, or even more than one, additional technique seems essential. Such a technique, or techniques, need not be considered an alternative but rather a complement in the difficult task of analysis for PAH compounds.

Shpol'skii spectroscopy (3,4) allied to a tunable dye laser excitation source appears to be one such additional technique. It offers the convenience of "line-like" spectra whose wavelength position can be used for identification and whose intensity can be used for quantification. We have been using such a system for several months to investigate its suitability for the analysis of atmospheric samples obtained from a Hi Vol sampler.

SHPOL'SKII SPECTROSCOPY

Atomic absorption or emission spectra are routinely used to analyse samples for such elements as lead, tin, nickel, etc. The sharp, isolated spectral features of such elements permit unambiguous identification of a given element, and the intensity of the features is a measure of the element's abundance. Granted, problems created by the 'interference' of other materials do arise, but these difficulties are not insuperable. Unfortunately, PAH compounds do not produce such "line-like" spectra in solutions at room temperature. These large molecules have a host of possible vibrational and rotational states, many of which overlap one another in wavelength (i.e. 'interfere'). Often such molecules are present as solutes in some organic solvent and this causes further 'interferences' due to the interactions between solvent and solute. Consequently room temperature spectra of solutions containing PAH compounds have broad features which make it impossible to identify which compounds are present in a complicated mixture obtained from ambient air (3,4). A different approach is needed for spectroscopic analysis.

The Shpol'skii effect provides a means for a new approach. PAH compounds, dissolved in a normal alkane at low concentration ($\leq 10^{-5}$M.), are cooled to

temperatures of several degrees Kelvin before their spectra are obtained. This has two important effects. Firstly, the PAH compound is rigidly located in the frozen solid. Hence it does not rotate. Moreover, it is in its ground state at this low temperature, i.e. minimum electronic, vibrational, rotational energy. The lack of rotation leads to vibrational levels within a molecule which are free of rotational structure. Secondly, the similarity in structure between the PAH solute and the normal alkane solvent allows the PAH molecule to "fit into" the matrix of the frozen solvent (6). Thus there are a small number of orientations of the structural axis of the PAH molecule realtive to the matrix of the frozen solvent. In turn, this leads to an equally small number of interaction energies between the solute and solvent.

As a consequence of these two effects, the spectra obtained from individual PAH molecules have a "line-like" character. This 'line-like' character arises because the individual vibrational energy levels of the molecule have no rotational structure to 'blur' their separation. Equally important, there are a small number of orientations of the solute relative to the solvent. Each orientation produces a shift in the set of energy levels of a PAH molecule, and so each orientation leads to a shift in the wavelength positions of the fluorescence. Were there a large number of orientations, the correspondingly large number of shifted wavelengths would blur into one another and so produce rather broad spectral features. Instead, there will be a small number of shifts which will lead to intense, discrete spectral features. In turn, that gives the sort of spectra needed for analytical spectroscopy.

EXCITATION AND EMISSION SPECTRA

Spectra from isolated PAH molecules have features which can be understood in terms of general ideas governing their absorption and emission properties. Since those ideas led to our strategy for the use of Sphol'skii spectroscopy in studying PAH compounds, a synopsis of them seems appropriate.

A common model for the absorption and any subsequent radiation by a large molecule is based on Kasha's view (7) that radiation occurs from the lowest energy level in any electronic state of a large molecule contained in a liquid or solid. This arises from fast (picosecond time scale) collisional relaxation processess between the molecule and its surroundings that leave the molecule in its lowest level of an electronic state. The excess energy is dissipated to its surroundings.

An examination of figure 1 will clarify this. Since the PAH molecule sits in a frozen solid at $15^{\circ}K$ (approximately), it absorbs radiation from the lowest vibrational level of its ground state (step 1). Clearly, the longest wavelength for this step will be $\lambda_0 = \frac{hc}{E_0}$. Following this excitation process the molecule relaxes to the lowest level of the singlet electronic state S_1 (step 2). From this lowest level it might undergo a crossing to the overlapping levels of the triplet electronic state T_1 (step 3B) or the singlet electronic state S_0. In the former event, there follows a rapid, non-radiative relaxation to the lowest level of T_1 (step 3). In the latter event rapid relaxation to the ground state of S_0 occurs. Whichever happens the molecule is now in the lowest level of the appropriate electronic state. If this state is above the ground state then a radiative transition can occur, either fluorescence (step 3A) or phosphorescence (step 4). A similar process follows an initial excitation into the second excited electronic state (S_2).
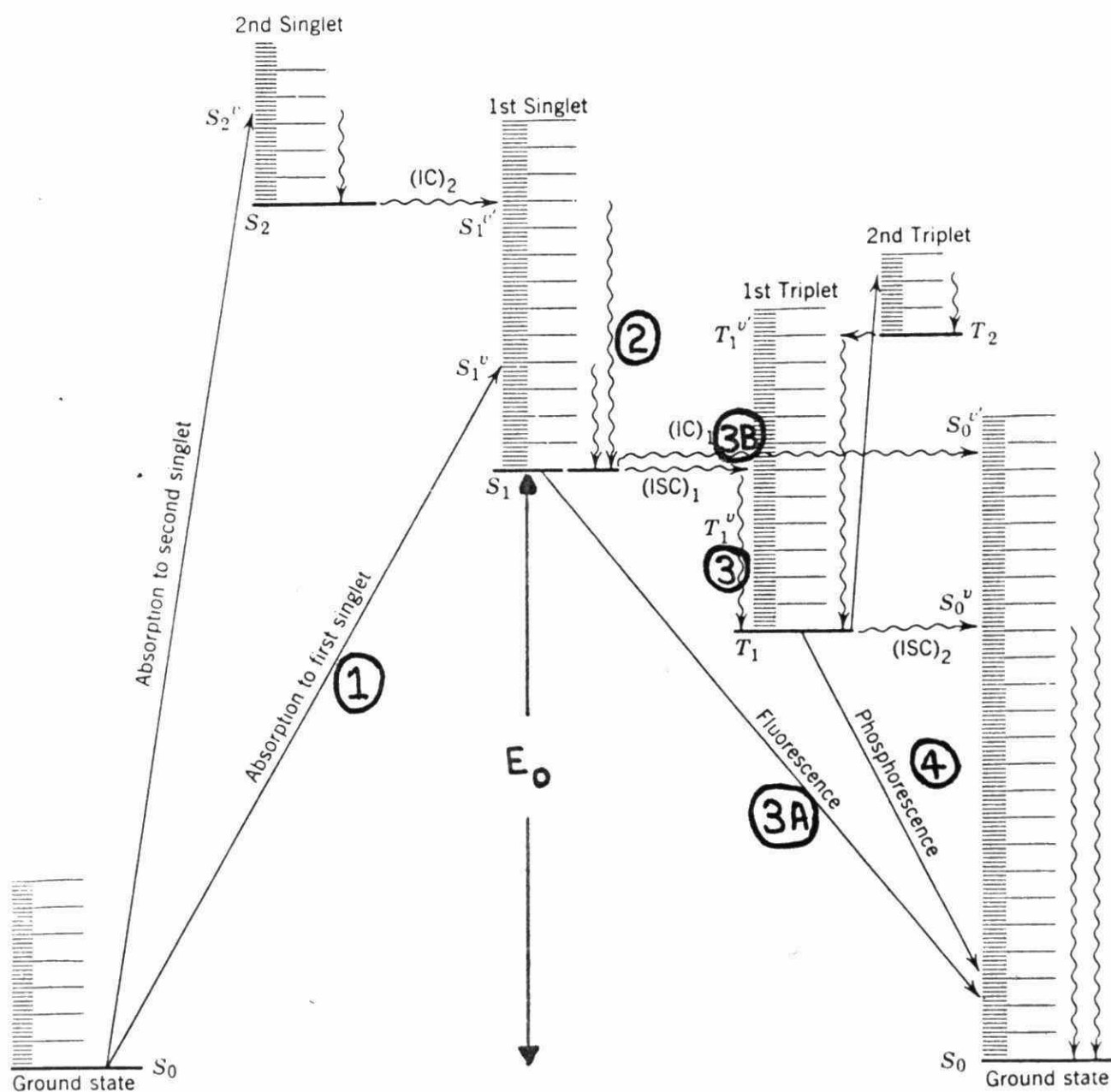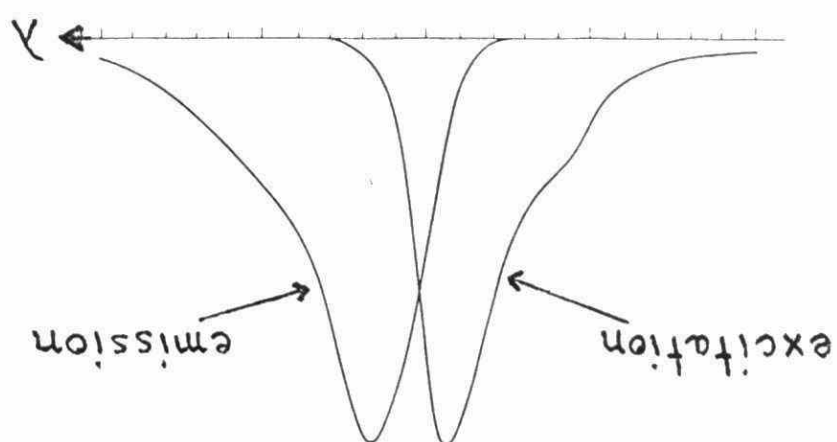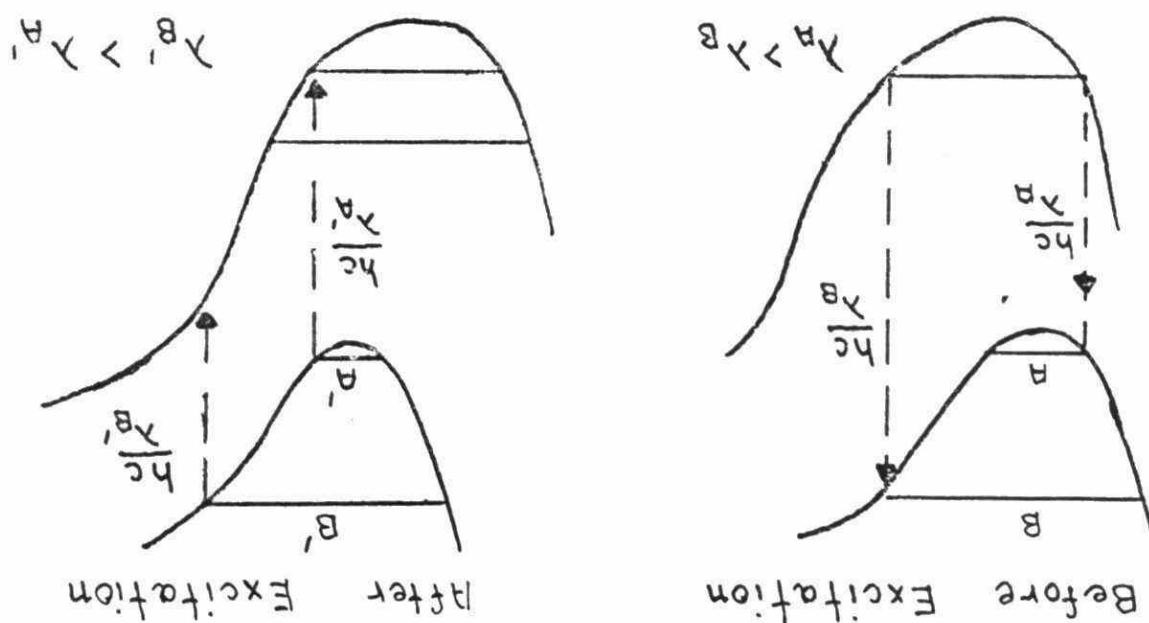
Figure 1   Energy Levels

As a consequence of radiative emission from the lowest excited singlet or triplet state all spectra for a given PAH molecule should have the same features regardless of the initial excitation wavelength (provided it is no larger than λ_O). However, some excitation wavelengths have a greater probability of absorption than others, and these excitation wavelengths will produce more intense fluorescence or phosphorescence. Hence a change in the wavelength of excitation will not change the spectral features in a spectrum but will change the intensity of those features.

Most large molecules undergo an increase in internuclear separation following excitation. The increased internuclear separation, along with the Franck-Condon principle for transitions between different rovibronic energy states, leads to a "mirror-image" relationship between absorption and emission spectra (see figure 2) from a given molecule (8). Figure 3 clarifies this point. The excitation wavelength λ_1 and λ_2 (absorption) become the fluorescence wavelengths λ_1' and λ_2' as a result of a changed internuclear separation in the excited state.

We used these ideas, particularly those coming from Kasha's rule, to develop the following strategy for our study.

1. Prepare laboratory solutions of pure, individual PAH compounds.
2. Irradiate a frozen sample.
3. Record the excitation spectrum.
4. Record the fluorescence spectrum.
5. Choose a combination of excitation and fluorescence wavelengths to selectively detect one PAH compound in the presence of others.
6. Apply the results of step 5 to an environmentally obtained sample.

EXPERIMENTAL PROCEDURE

Laboratory samples were prepared by weighing a few mg. of the PAH compound, (Aldrich Chemical or individually donated) dissolving it in normal Heptane (Caledon-193), and then diluting the stock solution to the desired concentration. Following this, a few ml. of the sample were placed in a small pyrex ampoule and de-gassed by alternate freezing to $77^{\circ}K$ (liquid nitrogen) and melting at low pressure while pumping. The ampoule was sealed under vacuum.

Environmental samples were obtained from Concord Scientific (Toronto) who had used a Hi Vol sampler in the Hamilton, Ontario area in the late winter of 1983 to obtain them (9). These samples came to us after extraction and subsequent elution by liquid chromatography. We used our basic sample preparation procedure (see foregoing paragraph), but incorporated three different solvent techniques. Firstly, we tried methanol as the solvent since the environmental samples had come to us in methanol. Secondly, we tried dilution of the methanol sample in normal heptane. Thirdly, we evaporated the sample to dryness and redissolved it in normal heptane.

A closed cycle helium refrigerator (CTI, model 21SC) was used to cool the sample contained in the pyrex ampoule. The ampoule was placed in a small copper block which was attached to the cold head of the refrigerator, the whole system being inside a vacuum chamber. Operating temperatures of $15^{\circ}K$ were typical, the temperature being measured with a calibrated silicon diode (Lakeshore Cryotronics, model DT 500). In practise the sample was plunged
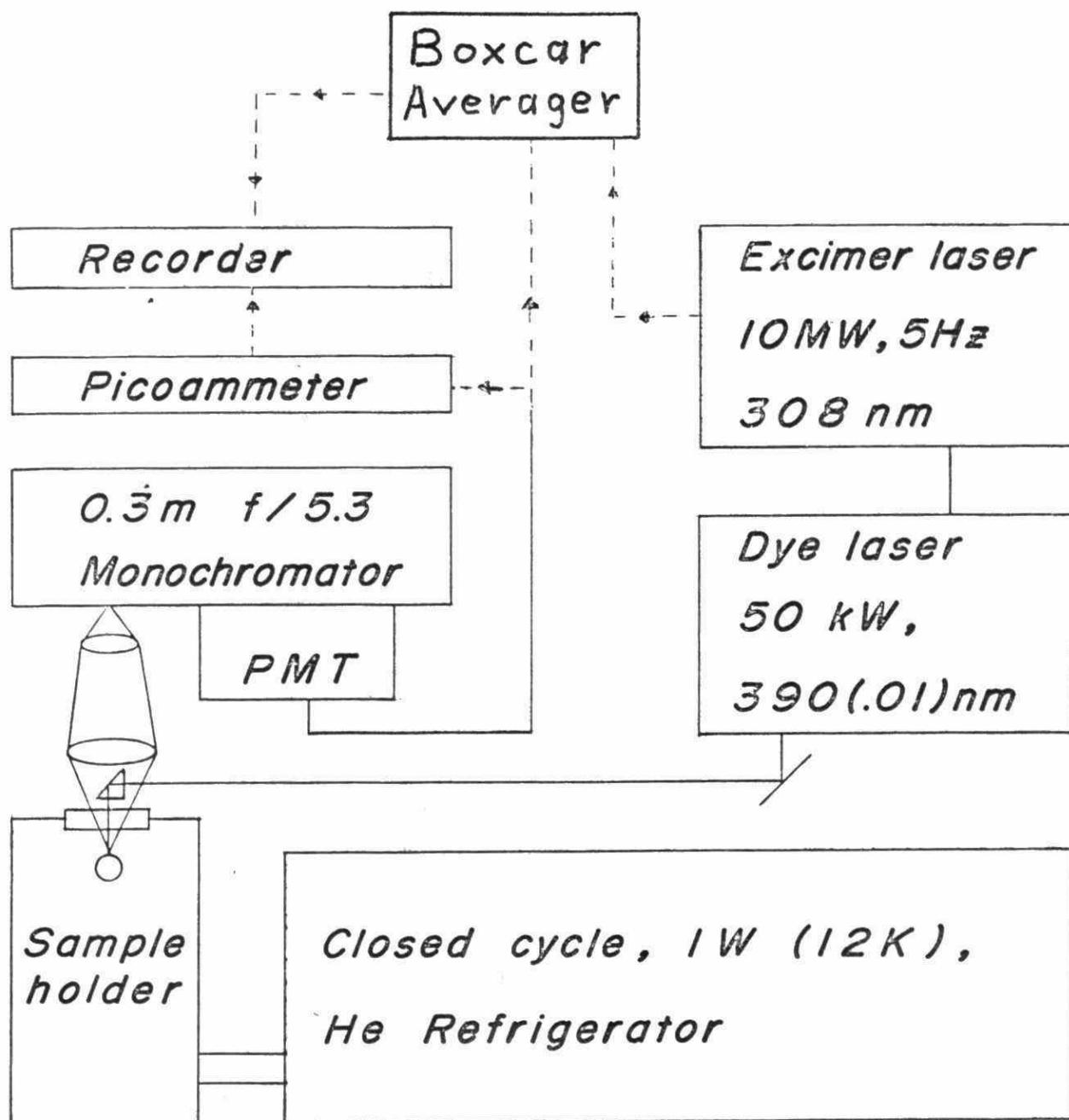
Figure 4   Equipment Schematic

into a Dewar of liquid nitrogen to 'quick-freeze' it before insertion into the copper block which was at a temperature of 77°K. This procedure aided the formation of the polycrystalline structure needed for Shpol'skii spectroscopy (10).

A tunable dye laser (Lambda Physik model FL2000) pumped by an excimer laser (Lumonics model 260-2) was used as an excitation source (see figure 4). The dye laser radiation (0.01 nm. bandwidth) could be tuned from 400 nm. to 360 nm. Fluorescence or phosphorescence from the sample was collected by a lens and focussed onto the slit of a monochromator (McPherson, model 218). Dispersed radiation was detected by a cooled photomultiplier tube (EMI, model 6256S) and then directed to the electronic processing equipment. Either box car averaging (using Evans Associates modules 4141 and 4130) or an electrometer (Keithley model 610B) with R-C averaging was used to convert the current pulses into a voltage suitable for a chart recorder. Excitation specta (i.e. absorption spectra) were obtained by fixing the monochromator at a known fluorescence wavelength, and then recording the intensity of that fluorescence as the dye laser was tuned through its range. Fluorescence spectra were obtained by selecting a fixed excitation wavelength and then recording the output of the photomultiplier as the monochromator was scanned from 390 nm to longer wavelengths. An optical cut-off filter was used in front of the monochromator to reduce the amount of scattered laser light reaching the photomultiplier tube.

RESULTS

We have recorded many spectra under various conditions of laser energy, compound concentration, spectral range, sample cooling rate, and electronic signal processing. For discussion we have selected a small number of spectra to illustrate both the technique, and our progress towards the analysis of an environmental sample. Included are both excitation spectra (360 nm to 400 nm) and fluorescence spectra (390 nm to 500 nm). The former are analagous to absorption spectra, whereas the latter are emission spectra from the first excited singlet state of the molecule. As yet, we have not studied phosphorescence emission, i.e. radiation from the triplet state. None of the spectra have been corrected for temporal or spectral variations in laser energy, nor for spectral variations in the detector response.

Figures 5 and 6 show excitation and fluorescence spectra of benzo (ghi) perylene, and perylene, while figure 7 shows two excitation spectra of perylene which were recorded at different fluorescence wavelengths, 445 nm and 478.5 nm. Figure 8 shows two fluorescence spectra of pentacene obtained at two different excitation wavelengths, 369.71 nm and 389.96 nm. Spectra of benzo (ghi) perylene at three different concentrations are shown in figure 9. In figure 10 are shown the excitation and fluorescence spectra of benzo(a) napthacene in our standard excitation and fluorescence ranges, while figure 11 shows two excitation spectra from a sample of benzo(g) chrysene. Lastly figure 12 has two spectra of an environmental sample from Hamilton, Ontario. One of the spectra was obtained from a dilute solution in methanol, whereas the other came from a solution in heptane. A more complete discussion of these results follows.

$\lambda_{em} = 419.9\,nm$

$0.1\,\mu g/ml\,n\,C_7$

392   387   382

$\lambda_{ab}(nm)$ (excitation)

$\lambda_{ab} = 389.03\,nm$

$0.1\,\mu g/ml\,nC_7$

405   410   415   420   425
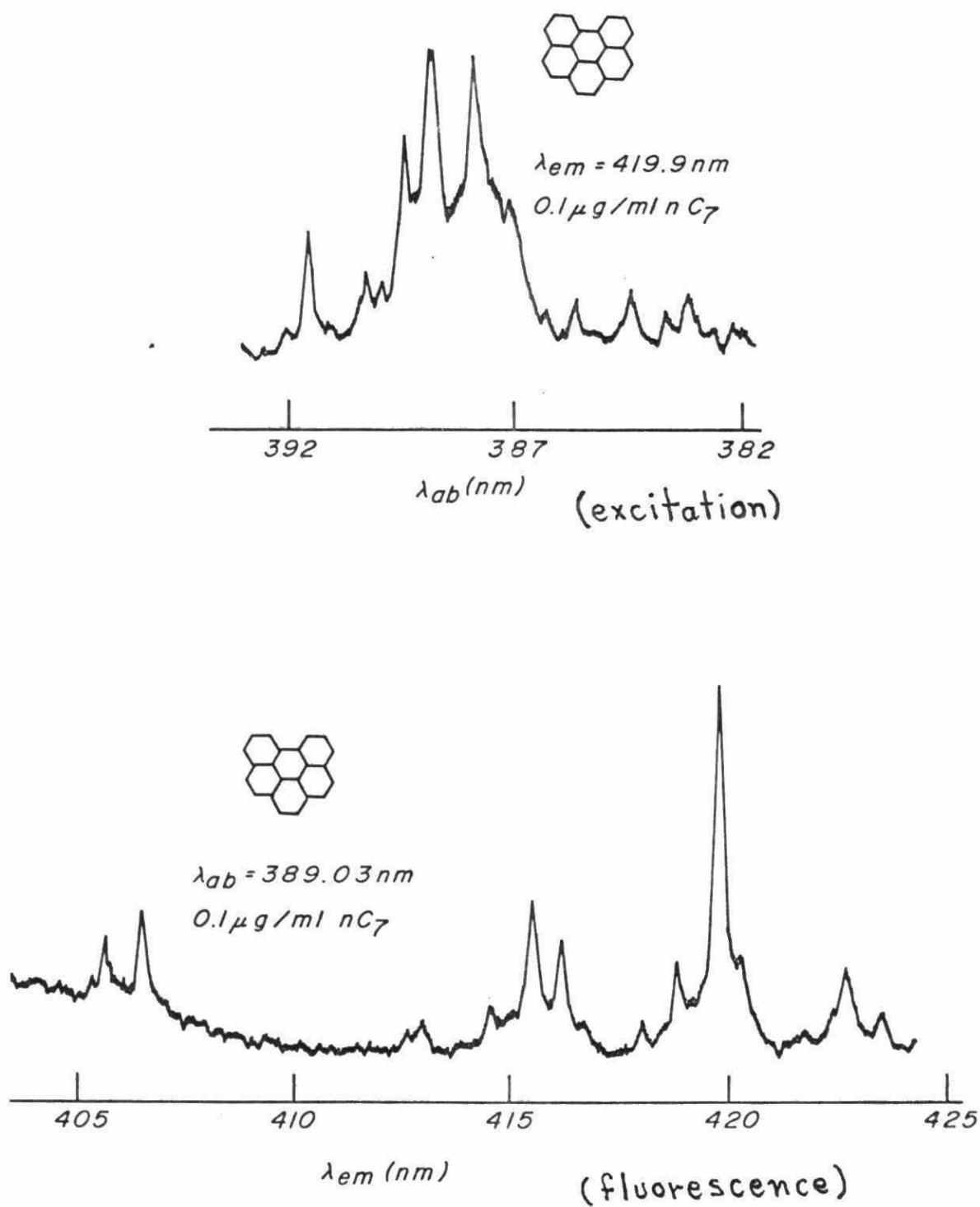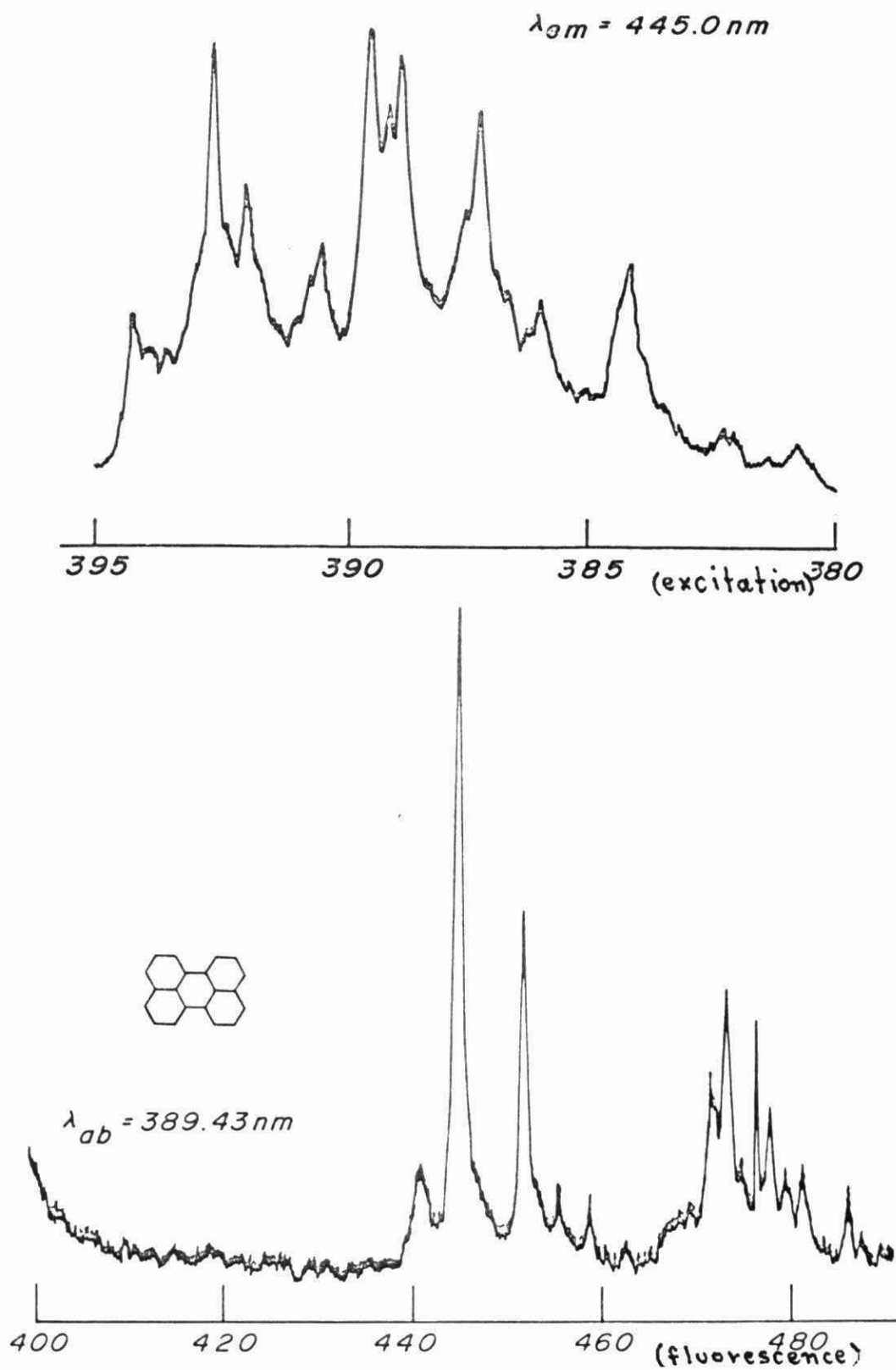
$\lambda_{em}(nm)$ (fluorescence)

Figure 5   Benzo(ghi)perylene

Figure 6   Perylene
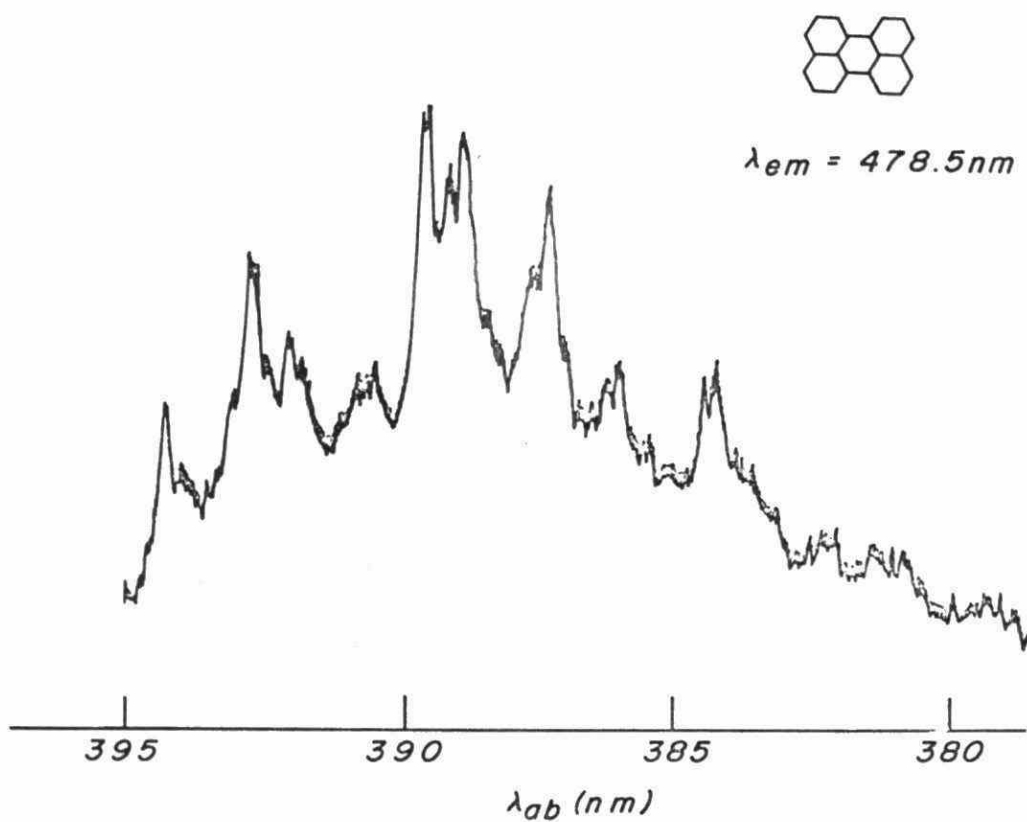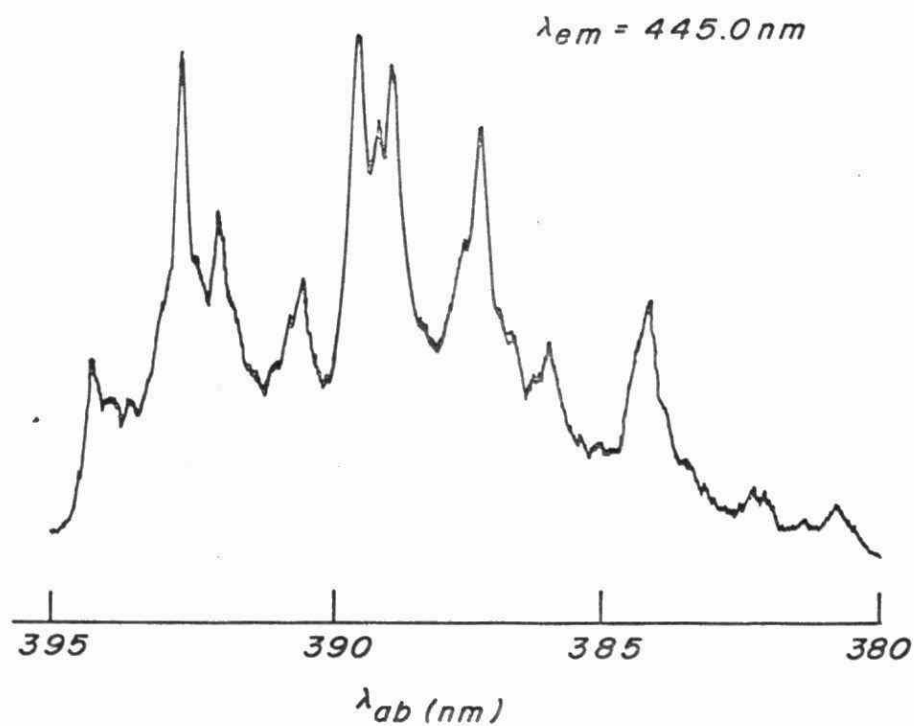
Figure 7 Excitation Spectra

λ 389.96

λ 369.71
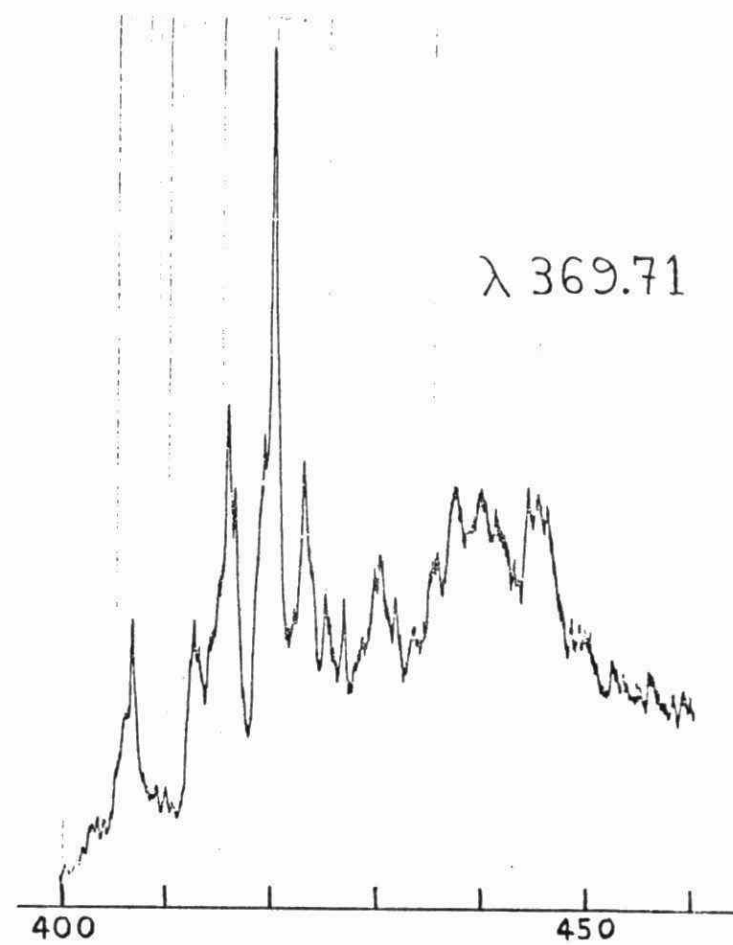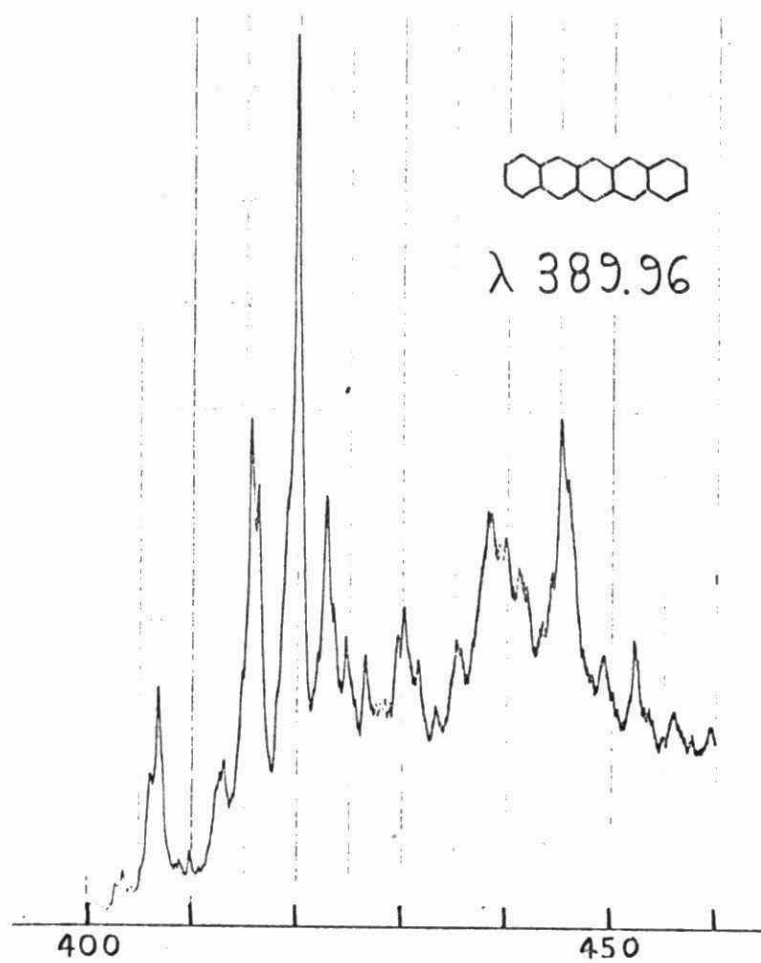
Figure 8 Fluorescence Spectra

DISCUSSION

The fluorescence spectra of benzo(ghi) perylene, and of perylene are clearly well separated from one another (figs. 5,6). Both compounds were on the list of six priority pollutants issued by the International Agency for Research on Cancer in the early 1970's. Their excitation spectra clearly show regions of overlap where a single excitation wavelength would jointly excite well separated fluorescence spectra from both compounds. Equally there are obvious locations where little overlap occurs and one compound's fluorescence spectrum could be preferentially excited. Both possibilities suggest approaches which might be useful in the analysis of these two compounds.

Figures 7 and 8 illustrate the implications of the Kasha rule for the spectra of PAH compounds in a Shpol'skii matrix. In figure 7 we see two excitation spectra of perylene, each of which closely resembles the other although the fluorescence wavelengths at which these absorption spectra were recorded differ substantially from one another. According to the Kasha model this behaviour can be expected since the appearance of any particular spectral feature will not depend on the wavelength of excitation although its intensity will. This is clearly evident from the two spectra in figure 7. Similarly, figure 8 shows two fluorescence spectra of pentacene which were excited at two very different wavelengths. These two spectra are essentially identical as we would expect on the basis of the Kasha model.

Quantitative determination of the amount of a PAH compound present in a sample is important. Figure 9 shows spectra from benzo(ghi) perylene obtained at three different concentration levels. Although the vertical scale is arbitrary there is a linear relationship in the relative intensities of the most intense feature over this concentration range. Moreover, it is clear that the intensity of the most intense feature is still well above the background level at the lowest concentration, and we estimate that we ought to be able to obtain a useful spectrum for at least another order of magnitude reduction in the concentration. The background level is the major limitation on the absolute sensitivity of this method. We are currently examining the contributions of photomultiplier dark current and of phonon emission (11) to this background. Additionally, these three spectra indicate the effect of concentration on the sharpness of the quasi-lines from the Shpol'skii effect. The spectrum taken at the highest concentration ($\sim 4 \times 10^{-5}$ M.) shows broader features than do the other two. This is an example of an empirical result, likely connected with some sort of clumping of the solute, that indicates concentrations above 10^{-5} M. should be avoided.

The excitation and emission spectra from benzo(a) napthacene shown in figure 10 may seem puzzling on first glance. Little structure is present in the excitation spectrum whereas the fluorescence spectrum does have structure. Yet the "mirror image" phenomenon mentioned previously suggests that structure in the fluorescence spectrum ought to be reflected as structure in the excitation spectrum (and vice versa). Two possibilities exist. One, the excitation region chosen for the fluorescence feature is poorly suited for effective excitation of this compound. Indeed, there is a substantial gap between the onset of fluorescence at 450 nm and the longest wavelength for excitation, which is 400 nm. Unfortunately, the dye in our tunable dye laser was inefficient at wavelengths longer than 400 nm. In addition scattered laser light would be a substantial problem at excitation wavelengths longer than 400 nm. As a result we were not able to explore this point. A second possibility is that the molecule does not display a "mirror image" behaviour. Such molecules are known.

Figure 9 Spectra at 3 dilutions

Figure 9 Spectra at 3 dilutions
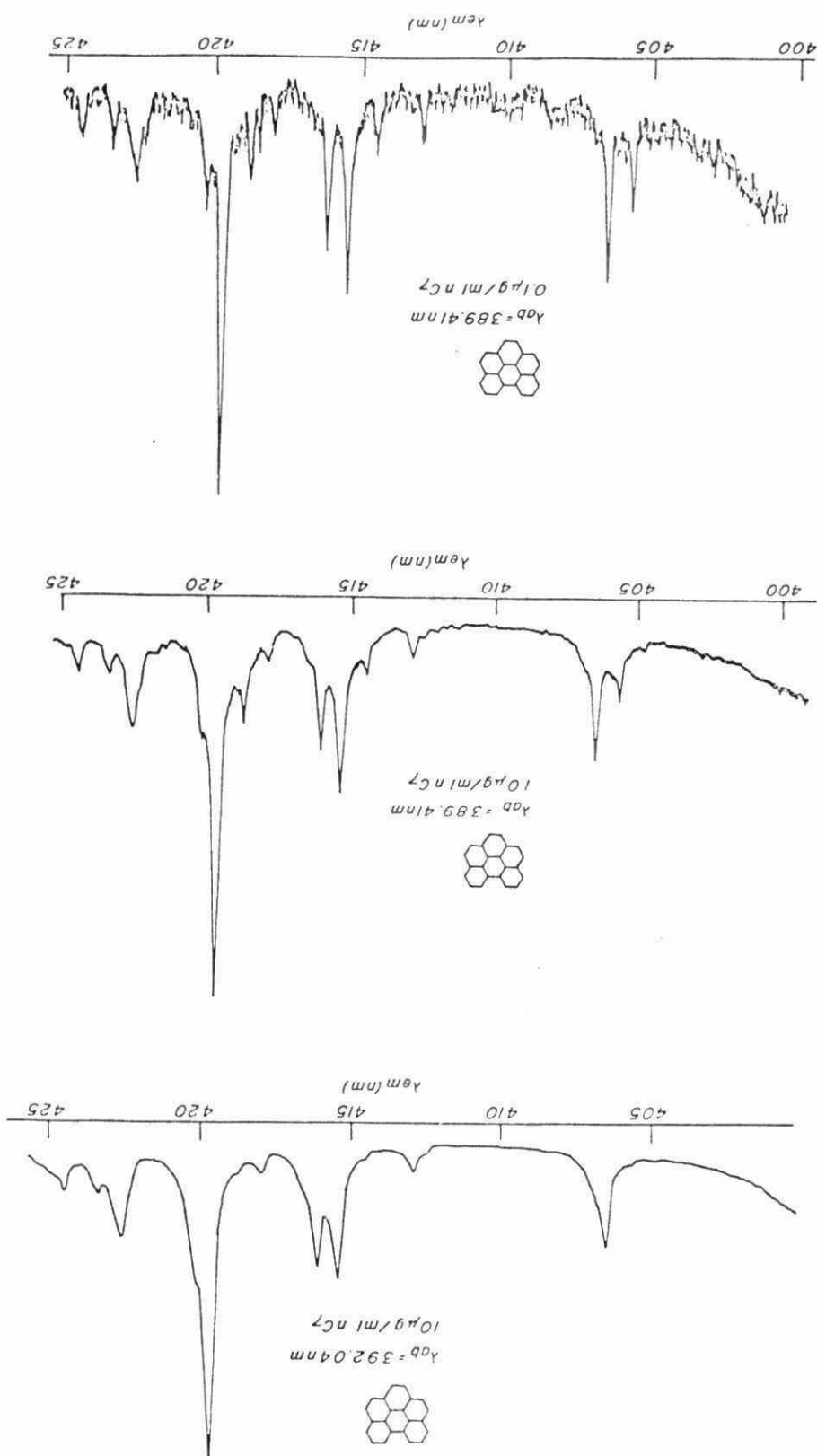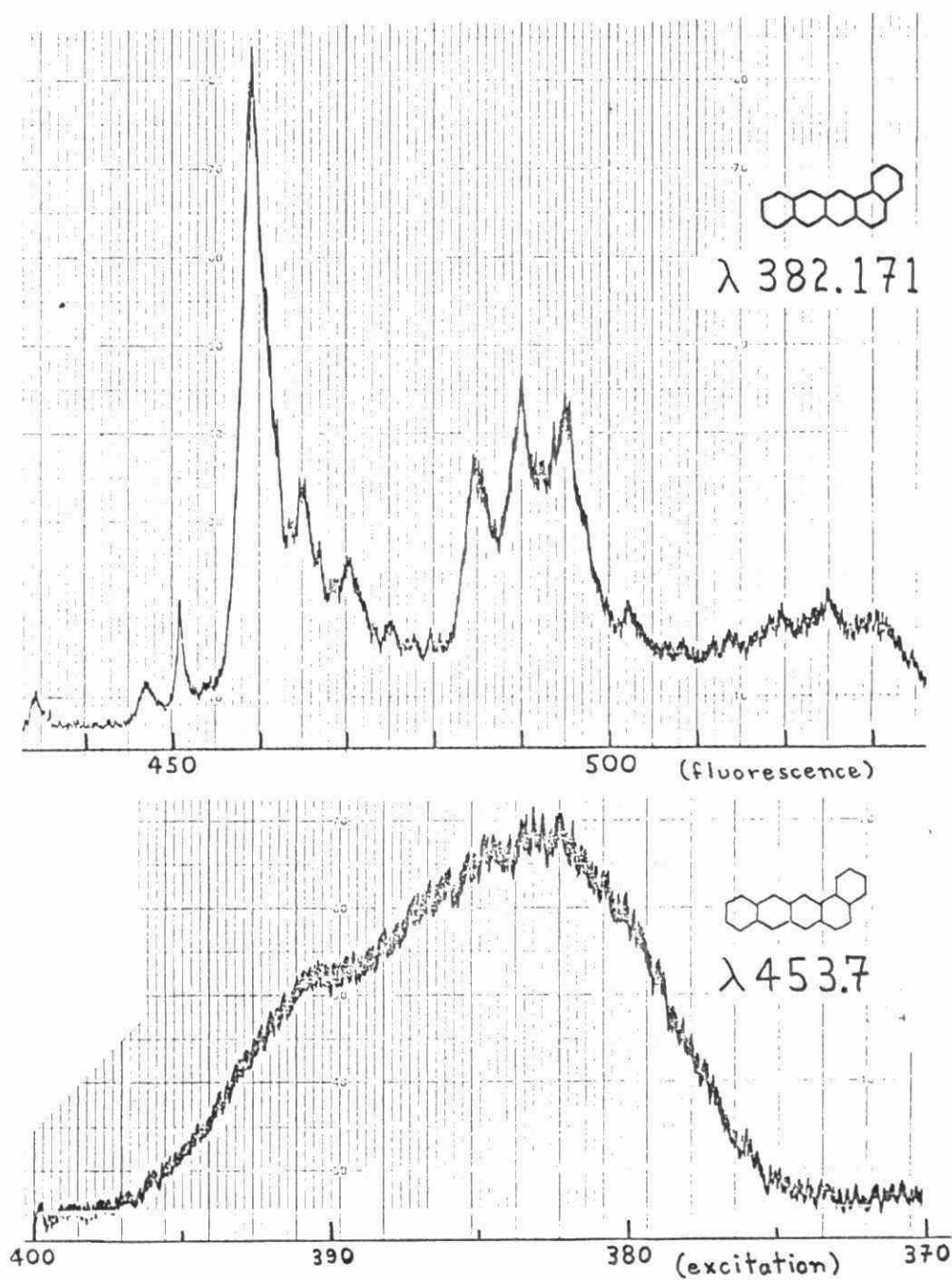
Figure 10   Benzo(a)napthacene
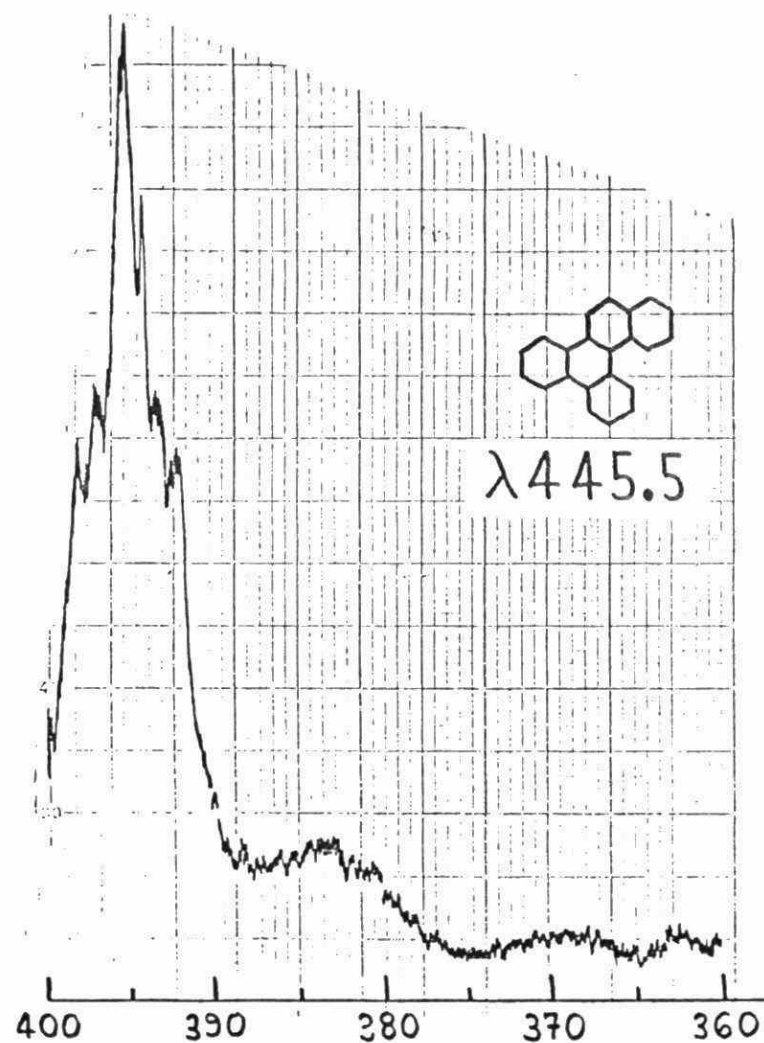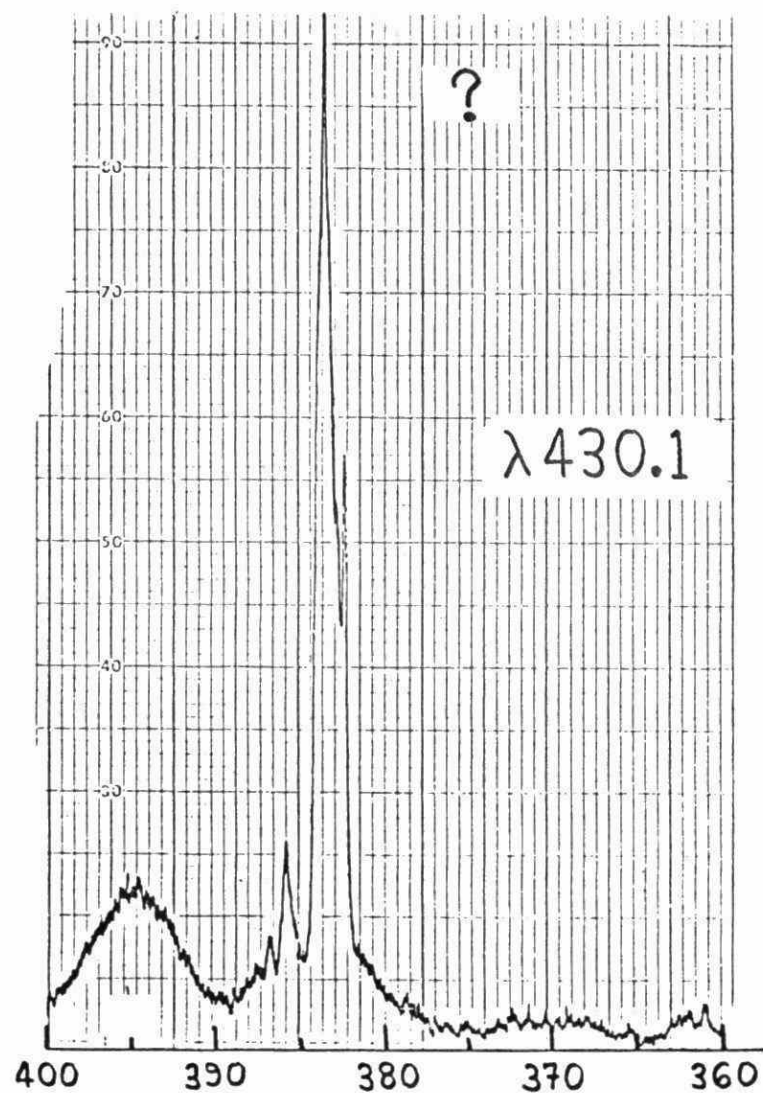
Figure 11   Excitation Spectra from Same Sample

Solvent Heptane

Solvent Methanol
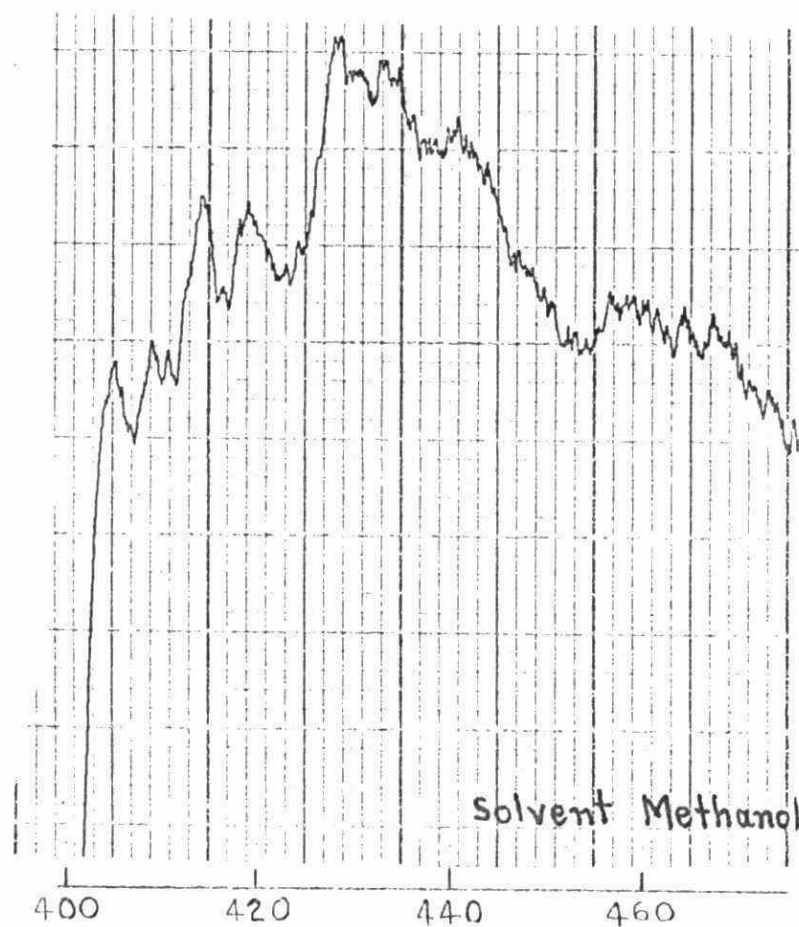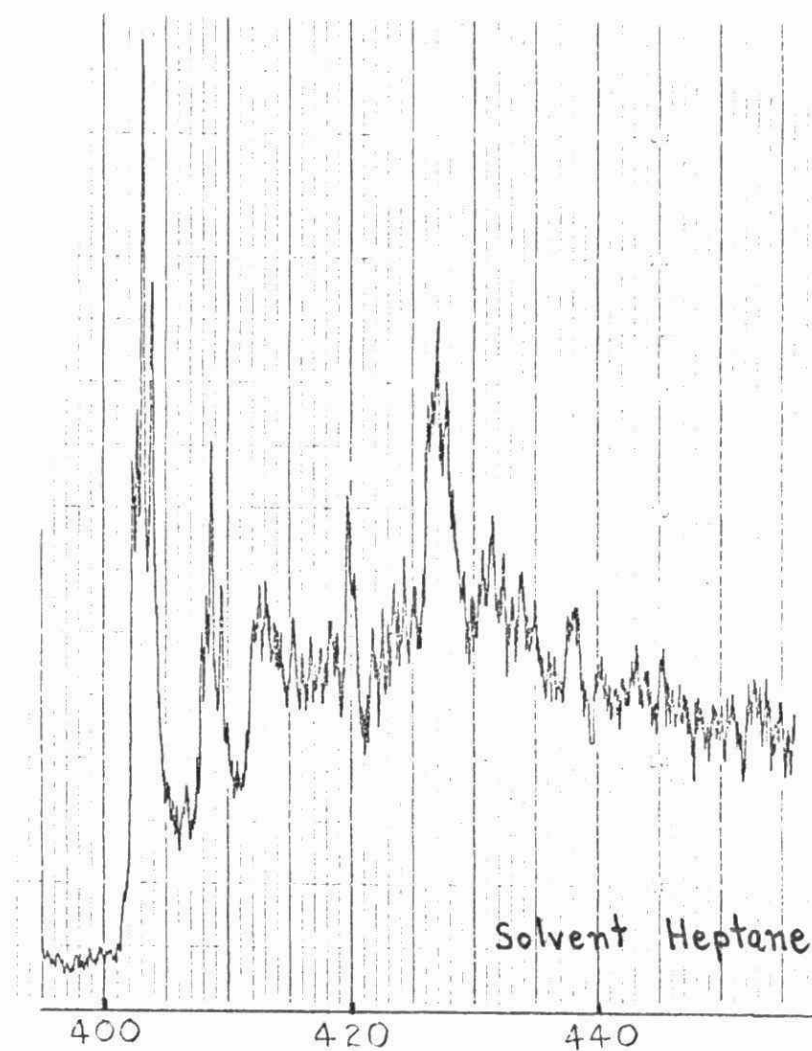
- 760 -

Figure 12   Environmental Sample

The two excitation spectra shown in figure 11 ostensibly come from the same sample, benzo(g) chrysene. However, they are very different even though they cover the same wavelength range. The Kasha model suggests they must then represent two different materials. Fluorescence spectra obtained at the major peak of the two different excitation spectra are also very different. Although we have not been able to identify the compound shown in figure 11(b), it clearly appears to be an impurity. At present its origin is uncertain. The usefulness of the Kasha model is apparent in this instance.

Figure 12 shows spectra obtained from an environmental sample. The spectrum, 12(b), in which the sample was dissolved in methanol has broad features with few sharp 'quasi-lines'. By contrast, that obtained with normal heptane as the solvent has many sharp features, several of which are attributed to benzo(a) pyrene. We are currently in the process of identifying the other PAH compounds present in this sample.

SUMMARY

An analytical procedure based on Shpolskii spectroscopy appears to be well suited to the detection of polycyclic aromatic hydrocarbons, their derivative compounds, and their heterocyclic analogues. Efforts at this method and integrating it into the standard repertoire of analytical techniques are well advanced in France (12), Sweden (4), and the United States (5). Proper use of the method will require a library of comparison fluorescence, excitation, and probably phosphorescence spectra. Additional spectra obtained with different solvents may be a necessity. Tunable dye lasers provide valuable excitation sources for PAH spectra because they are intense, have narrow spectral band widths, and can be pulsed for use in time delayed spectroscopy.

We have begun to build a small collection of spectra, concentrating on the six priority pollutants named by the International Agency for Research on Cancer; and we have begun to confront the problem of applying the technique to an environmental sample of unknown composition, which may consist of a complex mixture of PAH's and other compounds.

ACKNOWLEDGEMENTS

REFERENCES

1. Handbook of Polycyclic Aromatic Hydrocarbons (1983), ed. A. Bjørseth, M. Dekker, Inc. (New York).

2. Proc. of the 8th Int'l. Symposium on Polynuclear Aromatic Hydrocarbons (1983), ed. A. Dennis and M. Cooke to be published.

3. Shpol'skii, E.V. (1959), Soviet Physics Uspekhi, 2, 378-392.

4. Colmsjö, A. (1981), The Practice and Utility of the Shpol'skii Effect in Chemical Analysis. Univ. of Stockholm (Stockholm).

5. Yeng, Y., D'Silva, A.P., Iles, M., Fassel, V.A. (1981), Proc. SPIE - Int. Soc.

Opt. Eng., 286, 126-130.

6. Richards, J.L. and Rice, S.A. (1971), J. Chem. Phys., 54, 2014-2023.

7. Wayne, R.P. (1970), Photochemistry. Butterworths (London.)

8. Schafer, F.P. (1973), Dye Lasers. Springer-Verlag (Berlin)

9. Caton, R.B., Davis, C.S., Guerin, S.G., Tam, W.C. (1983), to be published in Proceedings of 8th Int'l. Symp. on PAH.

10. Nakhimovsky, L. (1983) to be published in Proceedings of 8th Int'l. Symp. on PAH.

11. Rebane, K.K. and Khizhynakov (1963), Opt. Spectrosc., 14, 193-197

12. Lamotte, M. et al and Garrigues, P. et al (1983), to be published in Proceedings of 8th Int'l. Symposium on PAH.

SAMPLING AND ANALYSIS OF PAH DERIVATIVES IN URBAN AIR PARTICULATES

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INTRODUCTION

The chemical composition of urban air particulate matter is very complex, consisting of hundreds of organic and inorganic components. Over the last decade, many efforts have been directed to the determination of the levels of known carcinogenic compounds, particularly polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene, in the organic solvent extractable fraction. However, in recent years it has become clear from work with microbial mutagenesis assays and chemical fractionation techniques that there are many hazardous substances present that are still largely uncharacterized. Studies have shown that much of the mutagenic activity in air particulate extracts is associated with compound classes other than the PAHs and that much of the activity is of the "direct-acting" type (1). The term "direct-acting" applies to those substances that cause reversion of Salmonella test strains in the Ames microbiological reversion assay (2) in the absence of the microsomal fraction of rat liver. The latter provides the oxidative "activation" of many chemicals to the reactive intermediates that act as the ultimate mutagens or carcinogens.

Several studies have shown that nitrated and oxygenated polycyclic aromatic hydrocarbons (nitro-PAHs and oxy-PAHs) are probably the principle direct-acting mutagens in automobile exhaust and urban air particulate samples (3,4). The environmental significance of these mutagens is not yet fully established, however, due in part to the lack of analytical methods for their determination and in part to the fact that many of these compounds may be formed as artifacts during the sampling step. Recent studies have shown that some PAHs can react readily with filter media, nitrogen oxides, sulfur oxides, ozone, and gas-phase photochemical smog to form a variety of derivatives (5).

Our research group has been examining the environmental and toxicological significance of the nitro-PAHs. Out of this work we have developed a routine analytical method that is sensitive and selective for the determination of nitro-PAHs in environmental samples, such as diesel exhaust and urban air particulates (6). The key steps in the procedure are: (a) a clean-up scheme that isolates the nitro-PAHs from the other components in the sample, (b) screening and quantitation by capillary column gas chromatography with simultaneous flame ionization and nitrogen-selective detection, and (c) structural confirmation with gas chromatography-mass spectrometry. In addition we have synthesized a large number of standard compounds that have been characterized by retention indices, mass spectra, and UV-VIS and NMR spectra.

Currently, we are extending our clean-up method to a wider range of compounds, investigating the extent to which nitro-PAHs and other PAH derivatives are artifacts of the sampling procedure, and studying new methods of sampling. The latter work is being done in collaboration with researchers at Concorde Scientific who are presently involved with the Ministry in contract research on alternatives to hi-vol sampling for PAHs. This paper will detail the analytical methodology that we have developed and the talk will present our latest results.

METHODS

Methylene chloride, acetone and methanol were "distilled in glass" quality purchased from Caledon Laboratories Ltd. (Georgetown, Ontario). Hexane and isopropanol were "HPLC grade" purchased from BDH (Toronto, Ontario). Nitro-PAHs were prepared from their corresponding PAHs by treatment with fuming nitric acid in acetic anhydride at $25^{o}C$. Depending on the reactivity of the PAH, the amount of fuming nitric acid used had to be varied in order to get predominantly mono-nitration or di-nitration. $[^3H]$-1-Nitropyrene was prepared as described previously (7).

Diesel particulate samples were provided by Dr. I. Salmeen of Ford Motor Co., Detroit. Urban air particulate samples from the Hamilton area were provided by Dr. L. D. Pengally (McMaster University, Hamilton, Ontario). Samples were collected for 24 hours at an average flow rate of 47 cubic feet per minute on 8 X 10 inch glass fiber filters (Schleicher and Schull Inc., Keene, N.H.) using a high volume total suspended particulate sampler (General Metal Works, Clev., Ohio). Air particulate samples were extracted with methylene chloride overnight in a Soxhlet extractor. Following concentration to about 10 ml in a rotary evaporator, the organic extract was filtered to remove particulates (0.5 um teflon filter, Millipore Corp., Bedford, MA) and evaporated to dryness under a stream of nitrogen. The weight of the dried residue was then determined.

The extracted organics dissolved in 1 ml of CH_2Cl_2 were injected into a silica Sep-Pak (Waters Associates Inc., Milford, MA) that had been previously washed with 5 ml CH_2Cl_2. With CH_2Cl_2 used as the eluent, the first ml of eluent was discarded while the next 3.5 ml was collected and evaporated to 200-300 ul under a stream of nitrogen. This solution was injected directly onto a Sephadex LH-20 column (Pharmacia Fine Chemicals, Dorval, Quebec) previously equilibrated in and eluted with methanol/methylene chloride (3:1). The eluent was monitored at 254 nm. The aromatic fraction which eluted in the 15-25 ml range was collected and evaporated to dryness under a stream of nitrogen.

In the final clean-up step a normal phase semi-preparative HPLC with a 250 X 9.4 mm M9 PAC column (Whatman Inc., Clifton, NJ) with a hexane/isopropanol mobile phase was used. The sample was dissolved in 20 ul methylene chloride and 80 ul hexane prior to injection. Excessive amounts of methylene chloride could not be used since the separation of the PAHs from the nitro-PAHs is impaired. Both the PAH and nitro-PAH fractions were eluted from the M9 PAC column using 0.5% isopropanol/hexane at a flow rate of 6 ml/min. PAHs were collected in the 5.0 to 8.8 min range and

nitro-PAHs in the 8.8 to 18.0 min range. The nitro-PAH fraction could be further split into 2 to 3 ring nitro-PAHs (8.8 to 12.5 min) and 4 to 5 ring nitro-PAHs (12.5 to 18.0 min). Dinitro-PAHs and more polar materials may be eluted using a step gradient from 0.5% to 10% isopropanol/hexane at the 20 min mark and collecting from 18 to 35 min. Fractions were evaporated to dryness and dissolved in a known volume of acetone for the final GC analysis.

A Varian 3700 gas chromatograph equipped with a cold on-column injector and flame ionization (FID) and nitrogen-phosphorus (NPD) detectors was used for GC analyses. An effluent splitter provided simultaneous FID and NPD detection. Two fused silica capillary columns were used: a) 25 m X 0.31 mm I.D., 0.17 um OV-1 (Hewlett-Packard, Mississuaga, Ontario), and b) 15 m X 0.32 mm I.D., 0.1 um DB-5 (JW Scientific Inc., Orangevale, CA). All analyses were performed with a temperature program of 60° to 150° at 14°/min, followed by 4°/min to 320°.
An Apple II+ microcomputer (Apple Computer Inc., Cupertino, CA) equipped with a 12-bit analog-to-digital converter (Interactive Microware Inc., State College, PA) was used for data aquisition, storage and plotting. HPLC was performed on Spectra-Physics 8000 liquid chromatograph (Santa Clara, CA) equipped with a Beckman 153 UV detector (Irvine, CA).

GC-MS analyses were performed on a VG Micromass 7070F mass spectrometer (VG Analytical, Altrinchan, U.K.) equipped with a Varian 3700 gas chromatograph. Samples were chromatographed on the fused silica capillary columns described above with the same temperature program. The column was connected directly to the mass spectrometer and inserted to within 2 cm of the electron beam. Electron impact ionization with an electron energy of 70 eV was used. Data was acquired with a VG 2035 data system, either by scanning from 400 to 80 amu, at 1 sec/decade, or by performing selected ion monitoring on up to 7 ions with accelerating voltage stepping and a 100 msec sampling interval for each ion.

RESULTS AND DISCUSSION:

In the analysis of a specific group of compounds present at trace levels in complex environmental samples, an analytical clean-up scheme directed toward the isolation of these compounds can dramatically reduce interferences in the final analytical step. The use of very specific, sophisticated methods of analysis such as MS/MS, while very powerful, are not readily available to most analysts and are not usually suitable to routine analysis. Since nitro-PAHs are found at trace levels in environmental samples an efficient clean-up scheme capable of routinely isolating this compound class from the complex matrix would be beneficial. For these reasons we have developed a specific clean-up scheme to isolate nitro-PAHs from diesel exhaust and urban airborne particulate matter (see Figure 1).

Airborne particulate samples, collected on glass fiber filters were Soxhlet extracted in methylene chloride overnight. Ultrasonic extraction with methylene chloride was investigated but was found to yield only about 10% of the total weight of extractable organics. Following evaporation the methylene chloride extract was

Sample Extract
(Methylene Chloride)

FILTRATION
(Millipore, 0.45 μm Teflon)

Particulates

SHORT SILICA COLUMN
(Sep-Pak, Waters Assoc.)

Very Polar Compounds

SEPHADEX LH-20
(MeCl$_2$/MeOH, 1:3)

Aliphatics

Aromatics

SEMI-PREPARATIVE NORMAL PHASE HPLC
(Whatman M-9 PAC, 50 cm, IPA/Hex)

Fraction A   Fraction B   Fraction C
(PAHs)   (Nitro-PAHs)   (Dinitro-PAHs)
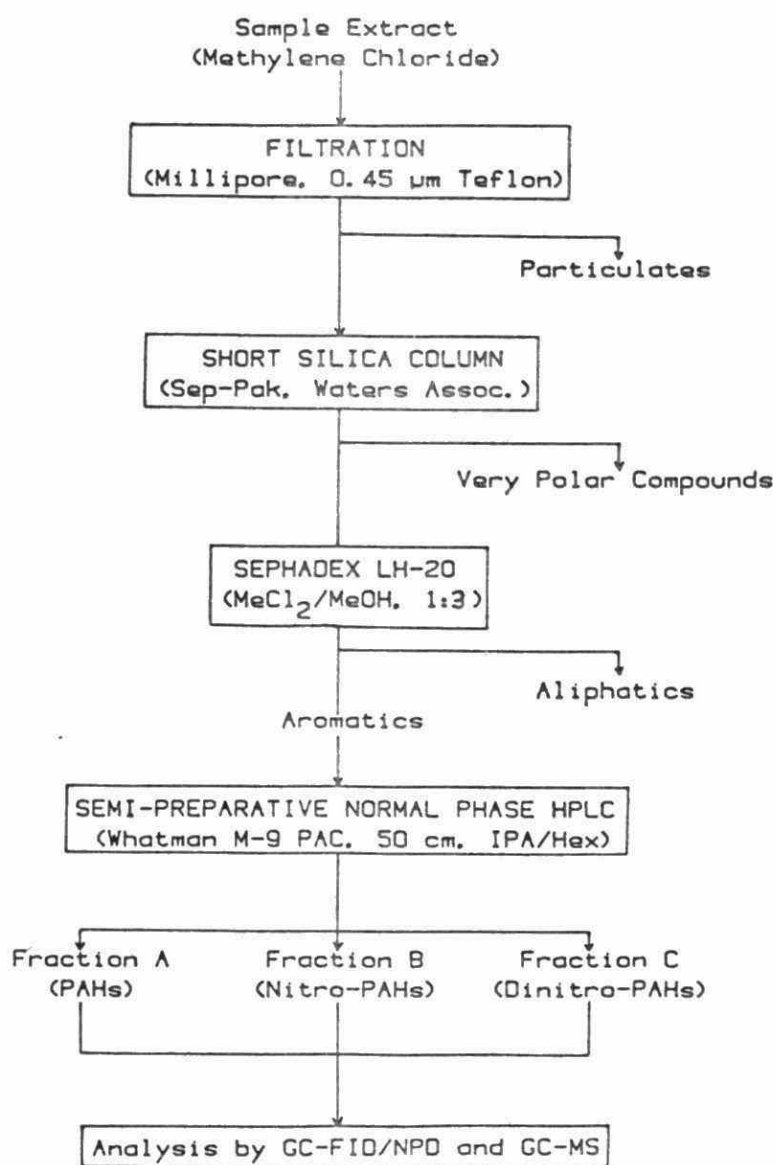
Analysis by GC-FID/NPD and GC-MS

Figure 1: Clean-up scheme developed for the isolation of PAHs and nitro-PAHs
in environmental samples.

filtered through a silica Sep-Pac to remove traces of particulates and highly polar compounds prior to the chromatographic steps where such compounds could interfere. In particular, with respect to the normal phase HPLC step, these compounds could lengthen analysis time, do irreversible damage to the column or cause memory effects.

The next step in the clean-up scheme was a class separation of aliphatics and aromatics using a Sephadex LH-20 column. A mobile phase of methylene chloride/methanol (1:3) was found to elute the aromatic fraction (PAHs, nitro-PAHs and other derivatives) as a relatively narrow band suitable for collection and concentration. The necessity of including this step was illustrated by its omission in the analysis of one air particulate sample. The resulting gas chromatogram was considerably more complicated, particularly with the presence of other nitrogen-containing compounds.

The final clean-up step involves normal phase HPLC, using a semi-preparative column with a mixed amino-cyano bonded phase, to separate nitro-PAHs from PAHs (see Figure 2). This is a desirable separation step because the PAHs are in far greater abundance than the nitro-PAHs and would interfere in both GC and GC-MS analyses. The advantages of using HPLC with a chemically bonded phase column rather than a conventional silica or alumina column include better reproducibility, higher resolution and less chance for irreversible absorption of trace components. A number of normal phase columns were investigated and it was found that the Whatman PAC (mixed amino-cyano) column afforded the best separation of PAHs and nitro-PAHs with a mobile phase of 0.5% isopropanol/hexane. If desired, the nitro-PAH fraction could be split according to ring size (e.g., 2-3 rings and 4-5 rings). In addition, it is possible to isolate another fraction containing dinitro-PAHs.

The clean-up scheme was tested for efficiency with tritiated 1-nitropyrene in the presence varying amounts of cold carrier, as well as sample matrix. An average recovery of 85% was observed over the nanogram to microgram range. This was considered to be adequate for trace analysis work. Typical front end loading for the clean-up scheme was in the order of 50 to 70 mg of extractable organics. It is estimated that the maximum capacity of the scheme as presented is at least twice this amount. The major problem is the exceedingly low solubility of the nitro-PAHs and the limited injection volume sizes that must be used to prevent deterioration of chromatographic efficiency. Larger column sizes would allow a greater capacity for the procedure.

A number of analytical finish techniques techniques have been evaluated. Reverse phase HPLC analysis with a UV detector was found to have considerable interference even after our clean-up, and confirmation of peak identity by mass spectrometry is difficult compared to GC-MS. We have found that GC-MS with selected ion monitoring, especially in the negative ion chemical ionization mode, offers very high sensitivity and selectivity for nitro-PAHs. However, it was not possible to justify dedication of our mass spectrometer to routine monitoring. We concluded therefore that capillary column gas chromatography with selective detection was the method best suited to routine screening of samples. Both electron capture and
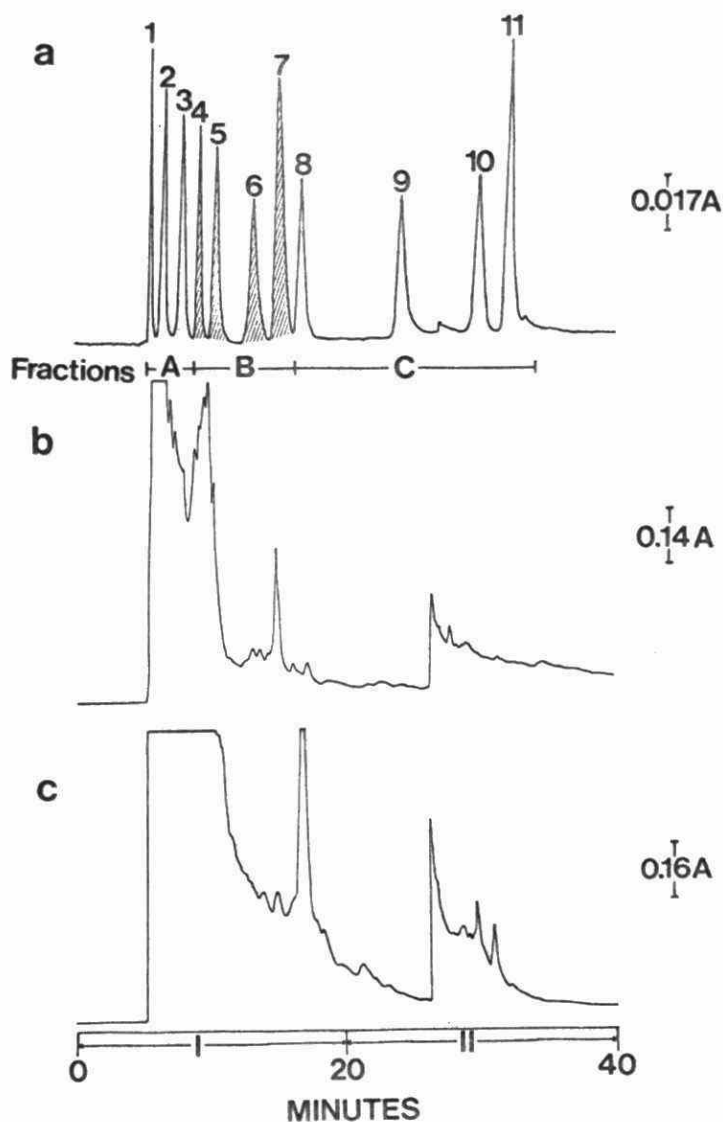
Figure 2: HPLC semi-preparative chromatography used in the clean-up scheme.
Chromatogram (a) illustrates the separation of a mixture of
standards (1 = naphthalene; 2 = pyrene; 3 = benzo[a]pyrene; 4 =
2-nitronaphthalene; 5 = 9-nitroanthracene; 6 = 1-nitropyrene; 7 =
6-nitrobenzo[a]pryene; 8 = 1,5-dinitronaphthalene; 9, 10 and 11 =
1,3-, 1,6- and 1,8-dinitropyrene). The other chromatograms
illustrate the separation of organics isolated from the Sep-Pak and
Sephadex LH-20 clean-up for: (b) a Peugot diesel exhaust particulate
sample, and (c) a Toronto airborne particulate sample. See Methods
for the conditions.

thermionic sensitive (nitrogen-phosphorous, NPD) detectors were investigated and it was determined that nitrogen selective detection provided the greatest selectivity.

The results that we report here were acquired with simultaneous flame ionization/nitrogen selective detection (FID/NPD). This method provides information on other components in the fractions analyzed and, in addition, allows accurate measurement of retention indices through co-injection of n-alkane standards. The use of cold on-column injection and fused silica columns was found to be essential for quantitation at trace levels. Flash injection techniques or packed columns showed poor detection limits for nitro-PAHs. Our GC-NPD analysis shows a detection limit of approximately 40 pg and linearity up to 50 ng for standards.

Retention indices for a large number of synthetic nitro-PAH standards have been measured. Many PAHs give several isomeric nitro-PAHs when nitrated, but the positions of substitution have been determined for only a few. Work in this area is continuing, as well as testing of the mutagenicity of different nitro-PAHs.

Some typical results for the analysis of nitro-PAH fractions of diesel exhaust and urban air particulate samples are illustrated in Figures 3 and 4, respectively. The identities of numbered peaks are given in Table 1. Nitro-PAHs were initially identified by retention indices and then confirmation was made by GC-MS. Only 1-nitropyrene was concentrated enough to give a good full electron-impact mass spectrum. The other nitro-PAHs were confirmed by selected monitoring of the molecular ions and M-30 fragment ions. Quantitation of these compounds was performed with the NPD detector, relative to an internal standard (1-nitronaphthalene). Other peaks appearing in the FID traces were identified by full electron-impact mass spectra to be oxygenated-PAHs and a phthalate. Although these cause interference in the FID trace, the NPD trace is not affected. The extent of clean-up is best illustrated by the fact that nitro-PAH peaks could not be observed in the chromatograms of the raw sample extracts prior to clean-up. They were masked by a large number of very intense peaks forming an "envelope" in the chromatogram. In addition, only one-hundredth of the material could be injected due to the presence of other compounds.

These methods are now being used to monitor the levels of PAHs, nitro-PAHs and oxy-PAHs in samples of airborne particulates that have been acquired with various types of sampling devices. The information from these experiments should be useful in determining the efficiency and degree of artifact formation with these different samplers.

REFERENCES

1. J.N.Pitts, Jr. et al., Toxicol. Letters 1, 65-70 (1977).
2. B.N.Ames et al., Mutat. Res. 31, 347 (1975).
3. D.Schuetzle et al., Int. J. Environ. Anal. Chem. 9, 93 (1981).
4. T.Nielson et al., Seventh Int. Symp. on PAHs, Columbus, Ohio, Oct. 1982.
5. J.N.Pitts, Jr. et al., Science 202, 515 (1978).
6. M.A.Quilliam et al., Seventh Int. Symp. on PAHs, Columbus, Ohio, Oct. 1982.
7. F. Messier et al., Carcinogenesis 2, 1007 (1981).
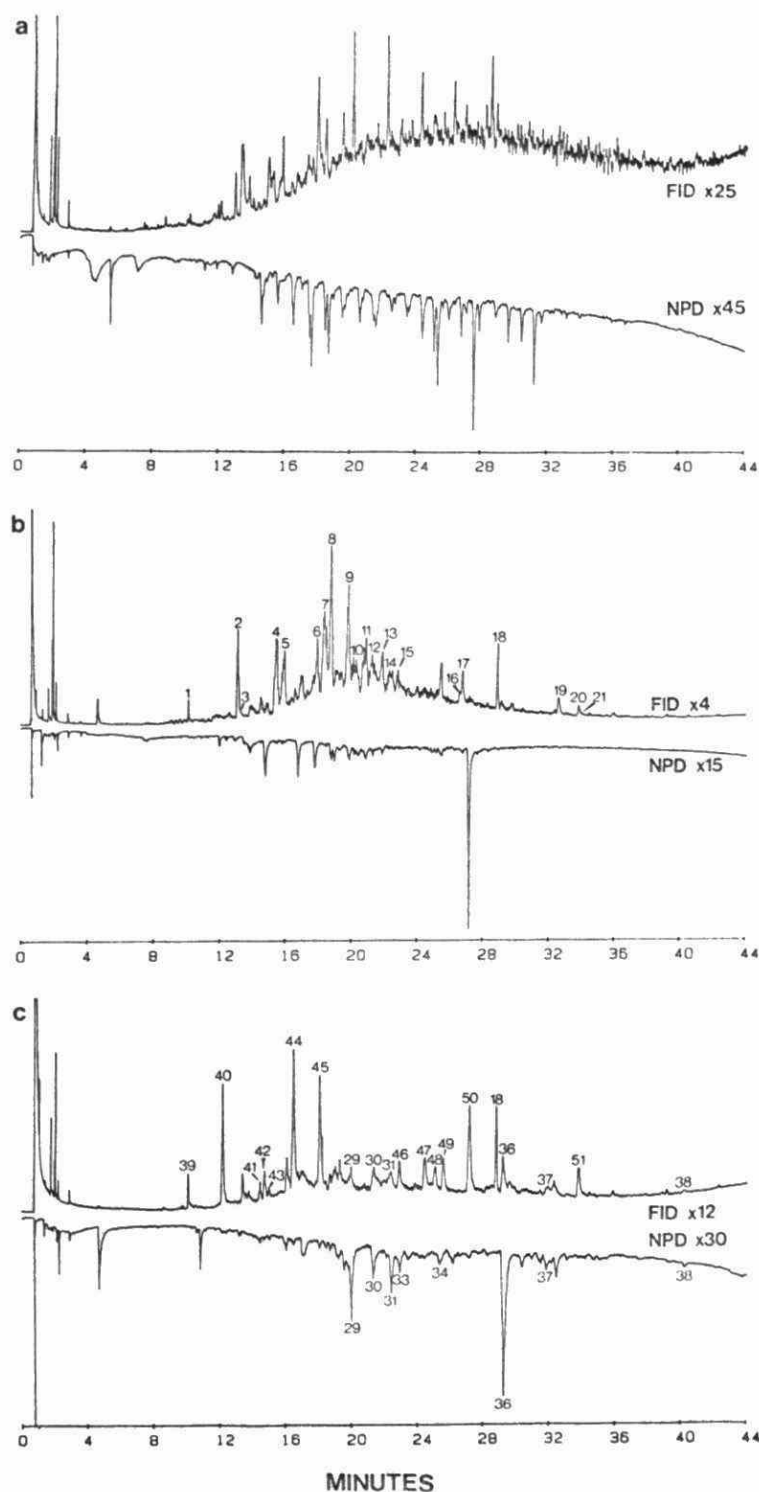
Figure 3: Capillary column GC analysis with simultaneous FID/NPD detection of
an Oldsmobile diesel exhaust particulate sample. Chromatogram (a)
is for the raw extract without clean-up, while chromatograms (b) and
(c) are for the clean-up fractions A (PAHs) and B (nitro-PAHs),
respectively. Column A (OV-1) was used with the conditions given in
Methods.
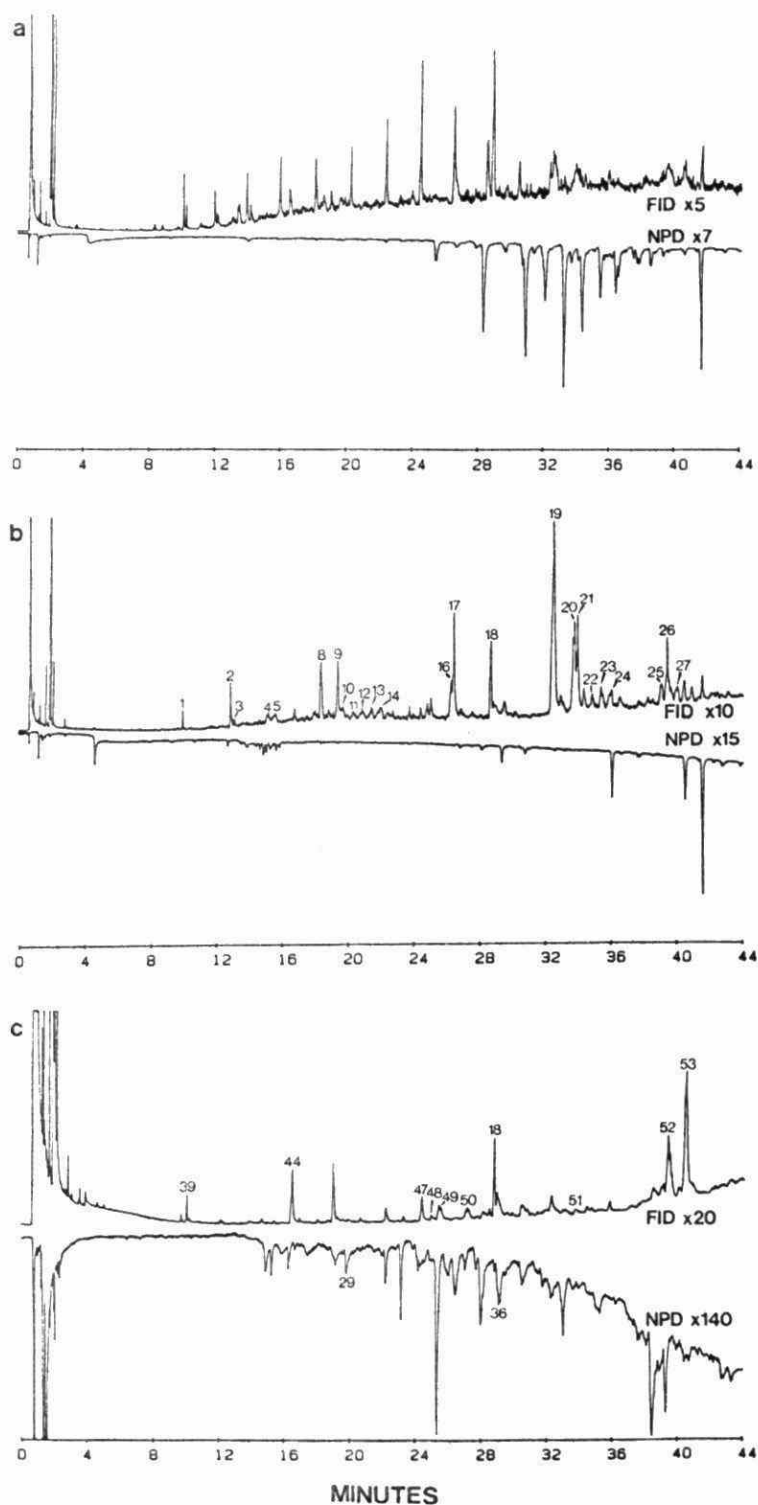
Figure 4: Capillary column GC analysis with simultaneous FID/NPD detection of
a Hamilton airborne particulate sample. Chromatogram (a)
is for the raw extract without clean-up, while chromatograms (b) and
(c) are for the clean-up fractions A (PAHs) and B (nitro-PAHs),
respectively. Column A (OV-1) was used with the conditions given in
Methods.

Table 1: Compounds identified in clean-up fractions A and B of diesel exhaust and airborne particulate samples

Peak No.	Mol Wt	Compound Name
1	166	Fluorene
2	178	Phenanthrene
3	178	Anthracene
4,5	192	Methyl(phenanthrene/anthracene)
6,7	206	C2-Alkyl(phenanthrene/anthracene)
8	202	Fluoranthene
9	202	Pyrene
10,11,12	220	C3-Alkyl(phenanthrene/anthracene)
13,14,15	216	Benzofluorenes or Methyl(pyrene/fluoranthene)
16	228	Benz[a]anthracene
17	228	Chrysene and/or Triphenylene
18	390	Dioctylphthalate
19	252	Benzofluoranthene
20	252	Benzo[e]pyrene
21	252	Benzo[a]pyrene
22,23,24	266	Methyl(benzopyrenes/benzofluoranthenes)
25	276	Indeno[1,2,3cd]pyrene
26	278	Dibenz[ah]anthracene
27	276	Benzo[ghi]perylene
28	223	a-Nitrophenanthrene
29	223	9-Nitroanthracene
30	223	b-Nitrophenanthrene
31-35	237	Nitromethyl(phenanthrene/anthracene)
36	247	1-Nitropyrene
37	261	b-Nitrobenzo[a]fluorene
38	297	6-Nitrobenzo[a]pyrene
39	222	Diethylphthalate
40	180	9-Fluorenone
41,42,43	194	Methyl(fluorenone/phenanthracenone/anthracenone)
44	208	9 10-Anthracenedione
45	204	4H-Cyclopenta[def]phenanthen-4-one
46	220	Methyl(phenanthrene/anthracene)-carboxaldehyde
47,48,49	230	Benzoanthracenones and/or benzofluorenones
50	230	7H-Benz[de]anthracen-7-one
51	254	Benzopyrenone
52	280	Dibenzofluorenone
53	304	Cyclopenta[ghi]picenone

APPLICATION OF BENZAMIDE DIRECTED METALATION STRATEGY IN SHORT AND REGIOSPECIFIC ROUTES TO PERI-METHYL SUBSTITUTED PAHs

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic pollutants which is widely dispersed in the environment. Recognition that this class of compounds plays a role in the epidemiology of occupationally-induced cancer has, in recent years, resulted in extensive research into the formation, occurrence, detection, chemistry and metabolism of PAHs. PAHs have been detected in the soil, in the atmosphere[1,2], in food[1,3], in water[4] and in tobacco and marijuana smoke[3,5]. Although there is evidence for the biosynthesis of PAHs by bacteria and higher plants[1], by far the major source of these environmental contaminants is from the combustion of fossil fuels.

Methylbenz[a]anthracenes (MBAs), PAHs that have been detected in cigarette smoke[6], stack gas and roofing tar extracts[7], have received much attention in studies concerned with structure-carcinogenic activity relation-ships[8]. Their presence in the environment emphasizes the need for the availability of synthetic samples, not only for their monitoring in the environment but also for continuing studies into the role that such PAHs play in the mechanism of carcinogenesis[9].

Apart from the extensive work of Newman[10], the availability of many of the monomethylbenz[a]anthracenes has been limited due to the complexity of the existing synthetic methods[11]. The evolving methodology of silicon protection in tertiary benzamide directed ortho lithiation[12] now allows its application to the synthesis of selected PAHs[13,14]. Details of this strategy[12] together with routes to selected peri-methyl substituted PAH precursors are herein reported.

RESULTS AND DISCUSSION

The regiospecific synthesis of polysubstituted benzene derivatives via the directed metalation of aromatic tertiary benzamides is well documented[15]. We have recently shown that the trimethylsilyl moiety can be used to effectively mask (a) the more reactive site to a carboxamide **1** and (b) the ortho methyl in an o-toluamide **2** to metalation reactions, thus allowing electrophile introduction into the alternate ortho positions (arrows in **1,2**) [12]. The ease of removal of the silyl groups[16] from the resulting products allows for rapid entry into diversely functionalized aromatic systems.

TMS = SiMe₃
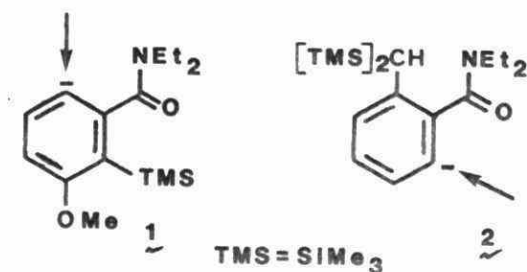
Representative of the use of silicon protection of the preferred 2-site in attaining a 1,2,5-trisubstituted aromatic pattern is the lithiation of **1** (**Scheme 1**) under the standard conditions[17] followed by quenching with selected electrophiles leading to substituted benzamides **3** in excellent yields. Subsequent desilylation using CsF in DMF/H_2O (10:1) at reflux for 8-20 h affords functionally useful aromatics **4** in good yields[18] (E = CHO, CH_3, $CONEt_2$).

Scheme 1.
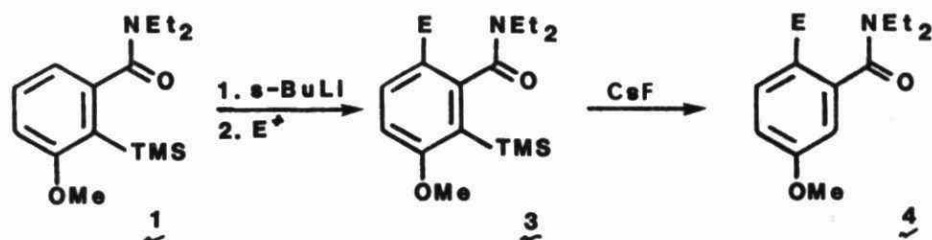
Scheme 2.
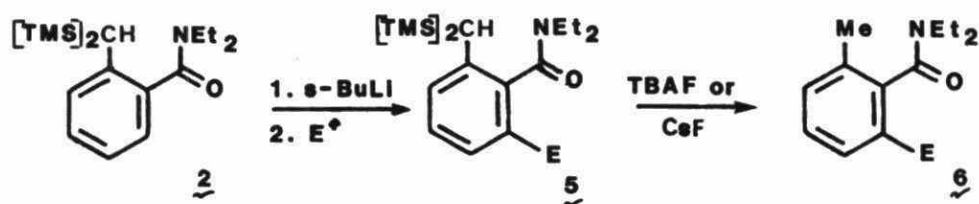
Compounds of the type 2 (**Scheme 2**) also undergo deprotonation under the standard conditions[17] to afford, after quenching with selected electrophiles, benzamides of the type **5** in excellent yields. Subsequent CsF or tetra n-butylammonium fluoride (TBAF)[16] mediated double desilylation affords 6-substituted-2-methyl benzamides **6** (E = $SiMe_3$, CH_3, CHO, SMe) in high yields. When the introduced electrophile is an aromatic aldehyde component, the silicon protection strategy in toluamide systems **2** allows for easy access to peri-methyl substituted PAH carcinogens[19] and naturally occurring anthraquinone natural products[20] (**Scheme 3**).

Scheme 3.
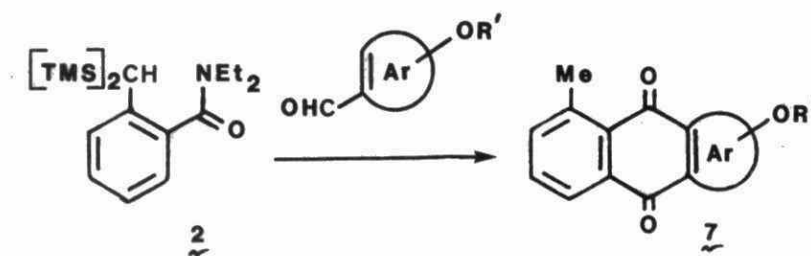
Thus metalation of the bis-trimethylsilyl o-toluamide **2** [21] under the standard conditions[17] followed by sequential treatment with 2-naphthaldehyde, CsF(DMF-H_2O/reflux/9 h), and TsOH(PhMe/reflux/9 h) without purification of intermediates gave the phthalide **8a** in 52% yield. Hydrogenolysis (CuSO$_4$ activated Zn/10% NaOH/reflux/24 h)[22] provided the acid **9a,** which upon Friedel-Crafts cyclization (TFAA/HOAc/RT/9 h)[17] and oxidation (Na$_2$Cr$_2$O$_7$/HOAc/RT/9 h) afforded 11-methyl-7,12-benz[a]-anthraquinone **10a** in 31% overall yield from 2 . The conversion of **10a** into the highly carcinogenic 7,11,12-trimethylbenz[a]anthracene has previously been achieved[23].

By an identical sequence of reactions, but substituting 1-naphthaldehyde for 2-naphthaldehyde, the synthesis of the intermediate phthalide **8b** and hence 8-methyl-7,12-benz[a]anthraquinone **10b** was effected in 42% overall yield from **2.** Methods for the conversion of **10a** and **10b** into the corresponding PAHs are well documented[13]. The scope of this methodology was further exemplified by treating the lithiated species of **2** with 9-phenanthrene carboxaldehyde to afford, after subsequent desilylation and cyclization, the phthalide **8c** in 63% yield, an intermediate required for the preparation of 10-methyl-9,14-dibenz[a,c]anthraquinone **11.**

8a Ar = 2-Naphthyl
8b Ar = 1-Naphthyl
8c Ar = 9-Phenanthryl

9a Ar = 2-Naphthyl
9b Ar = 1-Naphthyl
9c Ar = 9-Phenanthryl

10a R' = Me, R² = H
10b R' = H, R² = Me

11
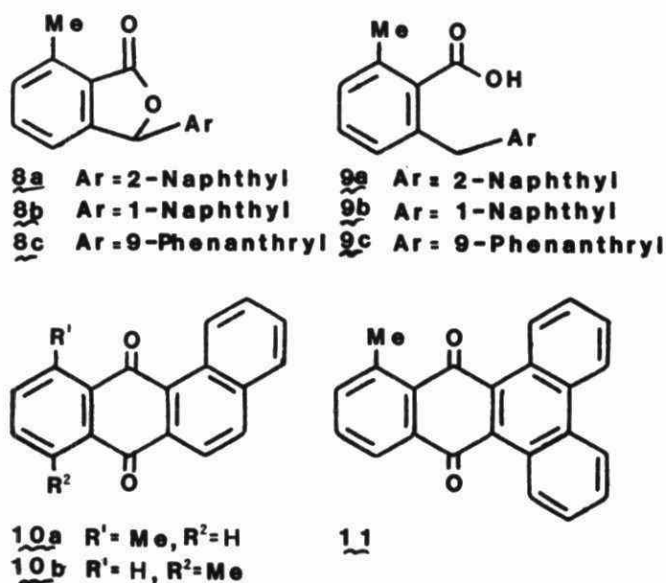
In light of recent work from our laboratory on the ortho N-aryl amination of tertiary benzamides[24], the above outlined silicon protection methodology will allow access to methyl substituted acridone systems and hence the corresponding AZA-PAHs.

In the exploratory example thus far investigated, the lithiated species of 2 **(Scheme 4)** was treated by oxidative coupling with the anilido-cyano cuprate 12 [25] to give the protected N-arylanthranilamide 13 in 48% yield. Subsequent desilylation using TBAF in THF afforded the methyl substituted N-arylanthranilamide 14 in 95% yield which was then cyclised to the corresponding 1-methyl acridone 15 in 50% yield[26] by refluxing in heptafluorobutyric acid for 36 hrs[24].

Scheme 4.
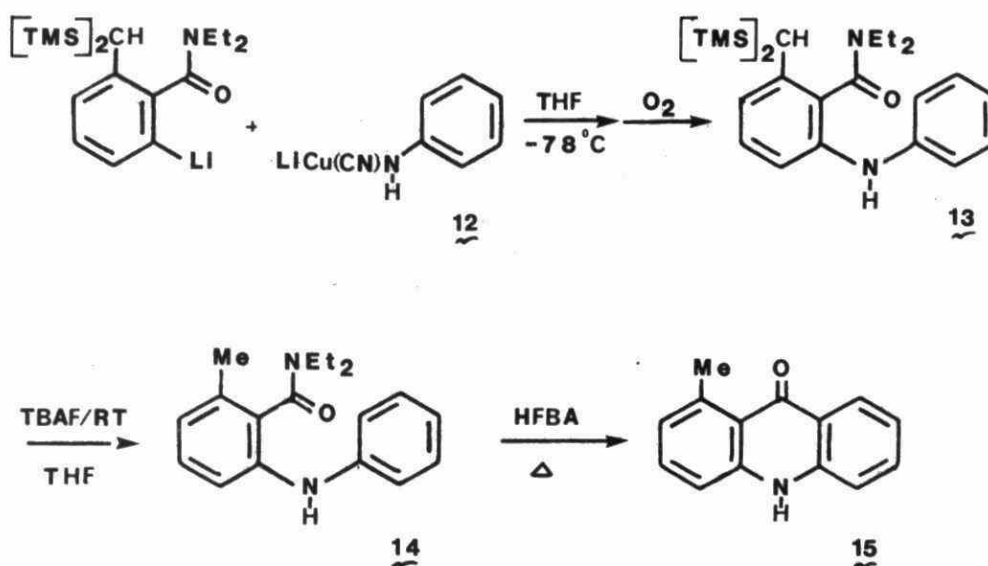
CONCLUSIONS

The tactic of silicon protection of ortho methyl functions has thus
allowed direct and brief routes to peri-methyl substituted PAH precursors.
Combined with the recently developed work in electrophilic ipso desilylation
in systems of the type 1 [27], a method that has hitherto received limited
application in synthesis[28], silicon protection in the directed metalation
strategy may, in the future, provide access to a variety of polysubstituted
PAHs. Work in this area is presently under investigation.

ACKNOWLEDGEMENTS

REFERENCES

1. Baum, E.J.: Occurrence and surveillance of polycyclic aromatic hydrocarbons. In: Polycyclic Hydrocarbons and Cancer, ed. H.V. Gelboin and P.O.P.Ts'o, Academic Press, New York, 1978, Vol. 1, pp 45-70.

2. Particulate Polycyclic Organic Matter: Biological Effects of Atmospheric Pollutants, National Academy of Sciences, Washington, D.C., 1972.

3. World Health Organization. 1973 Monograph on the Evaluation of Carcinogenic Risks of the Chemical to Man: Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds, Vol. 3, Geneva: Internat. Agency Res. Cancer, World Health Organization.

4. ZoBell, C.E.: Sources and biodegradation of carcinogenic hydrocarbons. In: Proceedings of the Joint Conference on Prevention and Control of Oil Spills, Washington, D.C., American Petroleum Institute 1971, pp. 441-451.

5. Lee, M.L.; Novotny, M.; Bartle, K.D. Anal. Chem. 48, 1976, 405-416.

6. Hoffman, D.; Schmeltz, I.; Hecht, S.S.; Wynder, E.L.: Tobacco Carcinogenesis. In: Polycyclic Hydrocarbons and Cancer, ed. H.V. Gelboin and P.O.P. Ts'o, Academic Press, New York, 1978, Vol. 1, pp. 85-117.

7. Thomas, R.S.; Lao, R.C.; Wang, D.T.; Robinson, D.; Sakuma, T.: Determination of polycyclic aromatic hydrocarbons in atmospheric particulate matter by gas chromatography-mass spectrometry and high-pressure liquid chromatography. In: Carcinogenesis, ed. R.I. Freudenthal and P.W. Jones, Raven Press, New York, 1976, Vol. 3, pp. 9-19.

8. Newman, M.S.: Carcinogenic Activity of Benz[a]anthracenes. In: Carcinogenesis, ed. R.I. Freudenthal and P.W. Jones, Raven Press, New York, 1976, Vol. 1, pp. 203-207.

9. Watube, T.; Ishizuka, T.; Isobe, M.; Ozawa, N. Science, **215,** 1982, 403.

10. Newman, M.S.; Gaertner, R. J. Am. Chem. Soc. **72,** 1950, 264.

11. Newman, M.S.; Khanna, J.M.; Lilge, K.C. Org. Prep. and Proc. Int. **11,** 1979, 271.

12. Mills, R.J.; Snieckus, V. J. Org. Chem. **48,** 1983, 1565.

13. Watanabe, M.; Snieckus, V. J. Am. Chem. Soc. **102,** 1980, 1457.

14. Doadt, E.G.; Iwao, M.; Reed, J.N.; Snieckus, V.: Synthesis of PAHs and AZA-PAHs using Aromatic Amide Directed Metalation Strategy. In: Polynuclear Aromatic Hydrocarbons, ed. M. Cooke and A.J. Dennis, Battelle Press, Columbus, Ohio, Seventh International Symposium, 1982, pp. 413-426.

15. Beak, P.; Snieckus, V. Acc. Chem. Res. **15,** 1982, 306: Snieckus, V. Heterocycles **14,** 1980, 1649.

16. Reiffen, M.; Hoffmann, R.W. Tetrahedron Lett. 1978, 1107.

17. de Silva, S.O.; Watanabe, M.; Snieckus, V. J. Org. Chem. **44,** 1979, 4802.

18. All new compounds show analytical and spectral data in agreement with the assigned structures.

19. Dipple, A.: Polynuclear Aromatic Carcinogens. In: Chemical Carcinogenesis, ed. C.E. Searle, American Chemical Society, Washington, D.C., 1976, pp. 245.

20. Thomson, R.H.: Naturally Occurring Anthraquinones, 2nd ed., Academic Press, New York, 1971.

21. Readily prepared in 92% yield in a one-pot sequence as follows: 1. 1 equiv s-BuLi/TMEDA/-78°C/45 min; 2. 1 equiv Me$_3$SiCl/-78°C/45 min; 3. 1 equiv. s-BuLi/TMEDA/-78°C/45 min; 4. 2 equiv Me$_3$SiCl/-78°C → rt/8 h.

22. Iwao, M.; Mahalanabis, K.K.; Watanabe, M.; de Silva, S.O.; Snieckus, V. Tetrahedron **39,** 1983, 1955.

23. Newman, M.S. *J. Org. Chem.* **48**, 1983, 3249. We express our gratitude to Professor Newman for providing a preprint of this paper and a comparison sample of **10a**.

24. Iwao, M.; Reed, J.N.; Snieckus, V. *J. Am. Chem. Soc.* **104**, 1982, 5531.

25. See reference 14 in ref. 24 of this paper.

26. Mp 312°C (MeOH) lit. mp 315°C, Gleu, K; Nitzsche, S. *J. Prakt. Chem.*, **153**, 200, 1939.

27. Mills, R.J.; Snieckus, V. *Tetrahedron Lett.* submitted for publication.

28. Colvin, E.; *Silicon in Organic Synthesis*, Butterworths, London, 1981, p. 125.

Synthesis of Polynuclear Aromatic Hydrocarbons of
Interest in Environmental Pollution.

A Seminar for Presentation at Technology Transfer
Conference No. 4. Nov. 29-30, 1983
by E. Lee-Ruff.

The abundance and toxicity of certain polynuclear aromatic hydrocarbons
(PAH) has been a matter of concern for a number of years[1]. The structural
characterization of these in minute quantities as is available in environ-
mental samples depend on the availability of well-defined reference stan-
dards. Furthermore the study of structure-activity patterns and understanding
of their molecular action of toxicity require the availability of closely
related model compounds. Recently certain PAH's having specifically a cyclo-
pentene ring fusion have been found to be widespread environmental contami-
nants at the same time exhibiting potent mutagenic activity. One such deri-
vative is cyclopenta(c,d)pyrene (1) which was recently characterized[2] and
shown to give a positive test in the Ames assay[3]. The interest in these
compounds arises from the unique structural features of 1 which has conspi-
cuously absent the "bay region"[4]. Current understanding of the mode of action
of these compounds in their toxic behaviour is that the PAH's are not the chem-
icals responsible but certain oxidized metabolites such as arene epoxides are
susceptible to binding with nucleophilic sites in cellular macromolecules thus
initiating the base-pair disruption mechanism and the onset of mutagenesis.
The PAH's which exhibit such activity all have a common structural feature of
having a "bay-region" as seen by the classical PAH benzo(a)pyrene[3] which is
metabolized by liver microsomes to benzo(a)pyrene diol epoxide 4. These parti-
cular bay-region diol epoxides are prone to nucleophilic addition reaction with
nucleophiles reacting at the epoxide function. In order to determine whether

other structurally related PAH's (those having cyclopentene ring fusion and similar oxygen heterocycles) are present in environmental PAH samples we
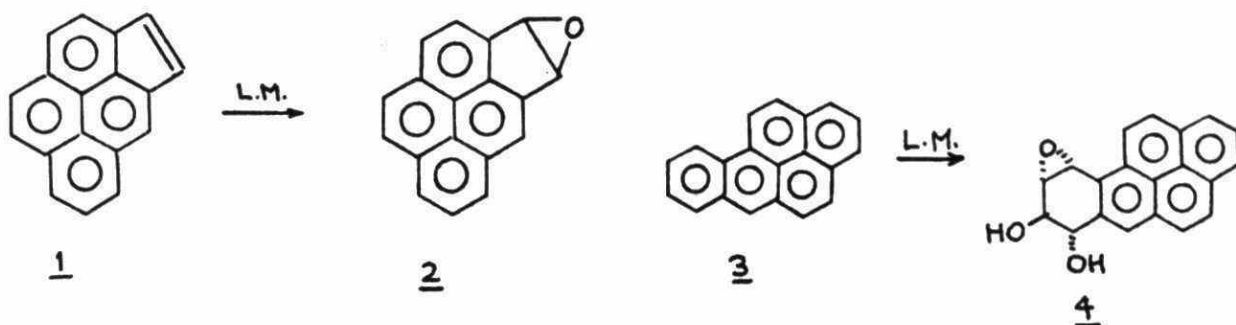
embarked on a program towards the synthesis of such derivatives. We had earlier developed a synthetic methodology for the preparation of polycyclic systems using cyclobutanones as intermediates[5]. Such a method yield PAH's incorporating such rings as cyclopentene, furans and pyrans, an example of which is shown below:

Scheme 1
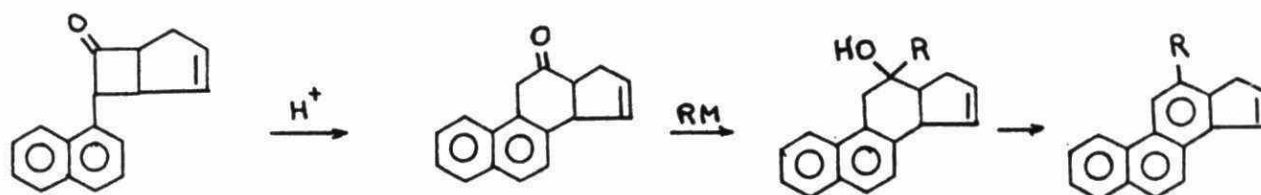
As is evident from this scheme it is possible to prepare specific alkylated PAH's by functionalizing the intermediate carbonyl containing compounds. Such compounds are of interest in that certain alkyl PAH's, specifically methyl
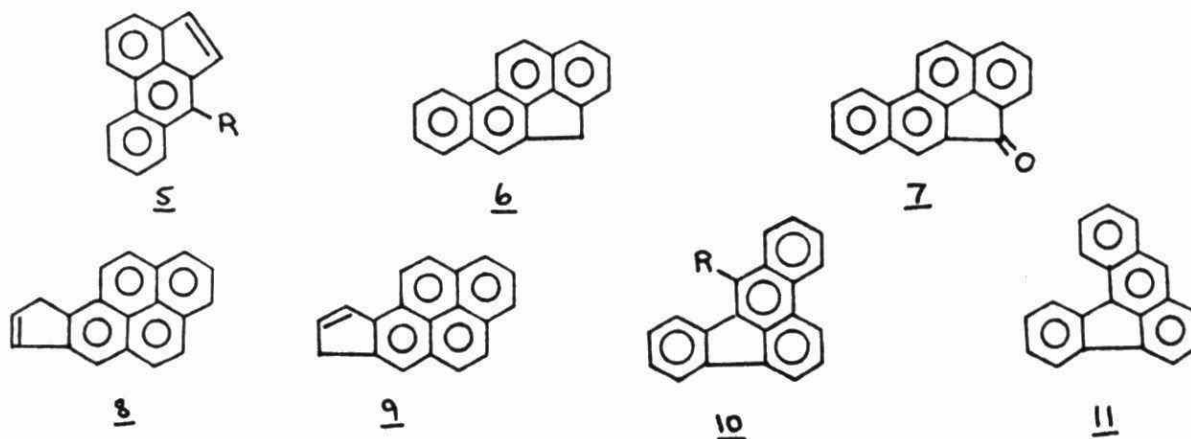
substituted PAH's, enhance the mutagenicity of these derivatives and are them-
selves found as environmental contaminants. The proposal was for the synthesis
of the **above** PAH's to be used as reference standards in the identification
of these in environmental samples. All of these compounds bear structural resem-
blance to either cyclopenta(c,d)pyrene and/or benzo(a)pyrene. Since our original
submission of this proposal compounds 8 and 9 have been prepared by Harvey[6]
and our original approach to 8 and 9 has been abandoned. We are reproducing
Harvey's methods for the preparation of 8 and 9. We have recently accomplished
the synthesis of 6 and 7 by the following scheme (2)[7]:

Scheme 2
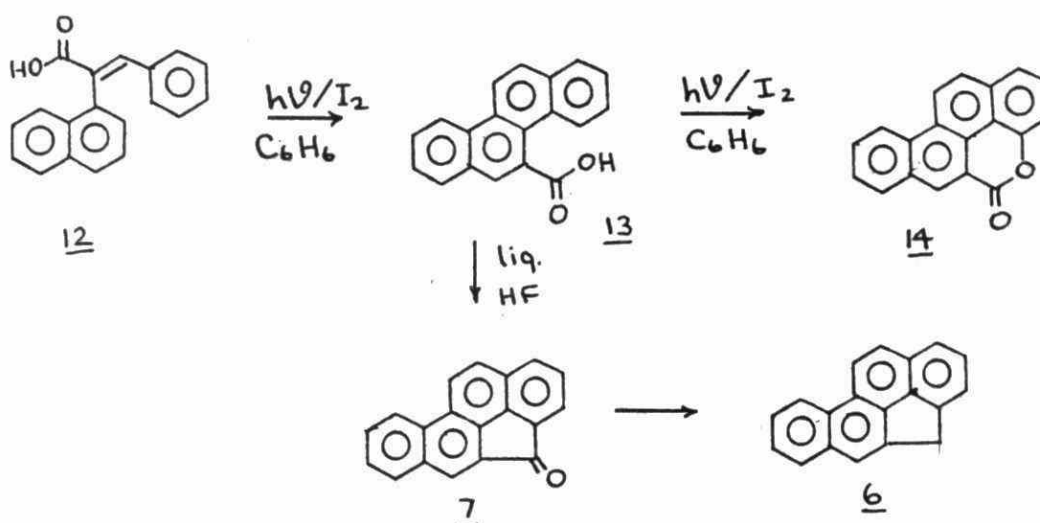
Photocyclization and oxidation of 12 gave chrysene-5-carboxylic acid and by-product
14 which was found to arise from secondary photolysis of 13. Lactone 14 has a
π-electronic configuration identical with benzo(a)pyrene. Its toxicity and
presence in environmental samples is currently under investigation. Cyclization
of 13 to 7 was accomplished using liquid HF. The ketone (7) was reduced to 6
by Wolf-Kishner reduction. The overall conversion yield was 30% for 6. This
particular synthesis is being reported in a forthcoming issue of the Journal
of Organic Chemistry[7].

The synthesis of 10 and 11 were accomplished using our route of cyclobuta-
none ring-expansion reaction. The scheme involves a divergent pathway using

the common cyclobutanone intermediate 15 which was obtained by cycloaddition

of fluorenylideneketene and 1,3-cyclohexadiene. Hydrogenation of 15 gave 16
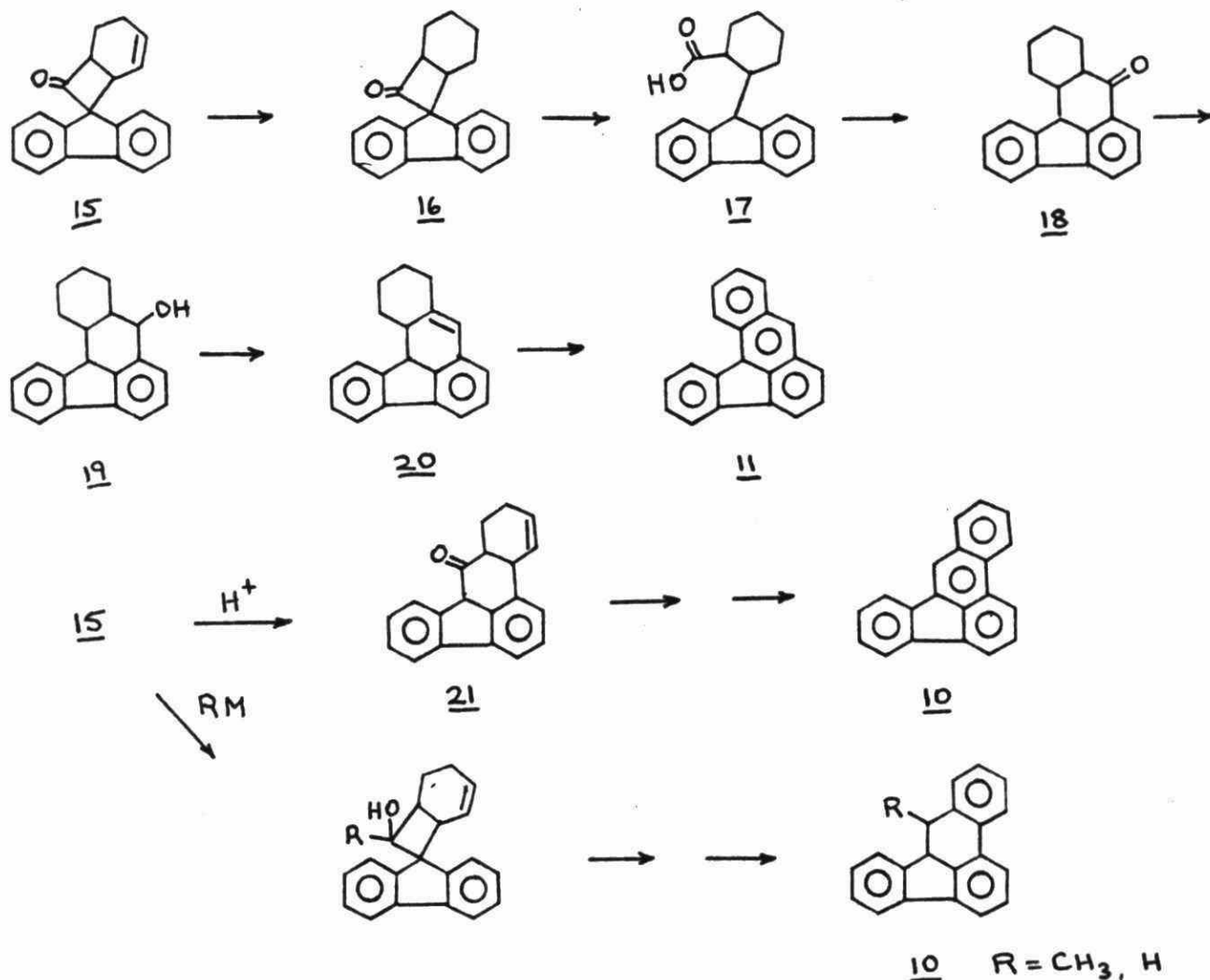
accompanied with over-reduced cyclobutanol. Ring opening of 16 or 15 with

hydroxide proceeded efficiently to give 17 and its unsaturated analogue respec-

tively. Hydrogenation of its unsaturated analogue produced 17 which could be

readily cyclized and dehydrated to 18 with liquid HF. Reduction of 18 with
LAH followed by acid dehydration and dehydrogenation with
DDQ gave benzaceanthrylene 11 which showed identical physical and spectral data

as reported recently for the same compound[8]. The same intermediate 15 could

be used for the synthesis of the angular-fused benzo(b)fluoranthene (10).

Acid-catalyzed rearrangement of 15 to 21 could be accomplished with methansul-

phonic acid. Reduction of tetracyclic ketone 21 with LAH followed by dehydra-

tion and dehydrogenation with DDQ gave the parent benzo(b)fluoranthene 10.

The methyl derivative $\underline{10}$ R = CH_3 could be prepared by way of methylation

of $\underline{15}$ with $LiCH_3$ to alcohol $\underline{22}$. Acid-rearrangement of $\underline{22}$ followed by dehydro-

genation with DDQ gave 5-methylbenzo(b)fluoranthene in 20% overall yield. The

acephenanthrylene derivatives $\underline{5}$ (R = H, CH_3, n-C_4H_9) were prepared by a similar

route.
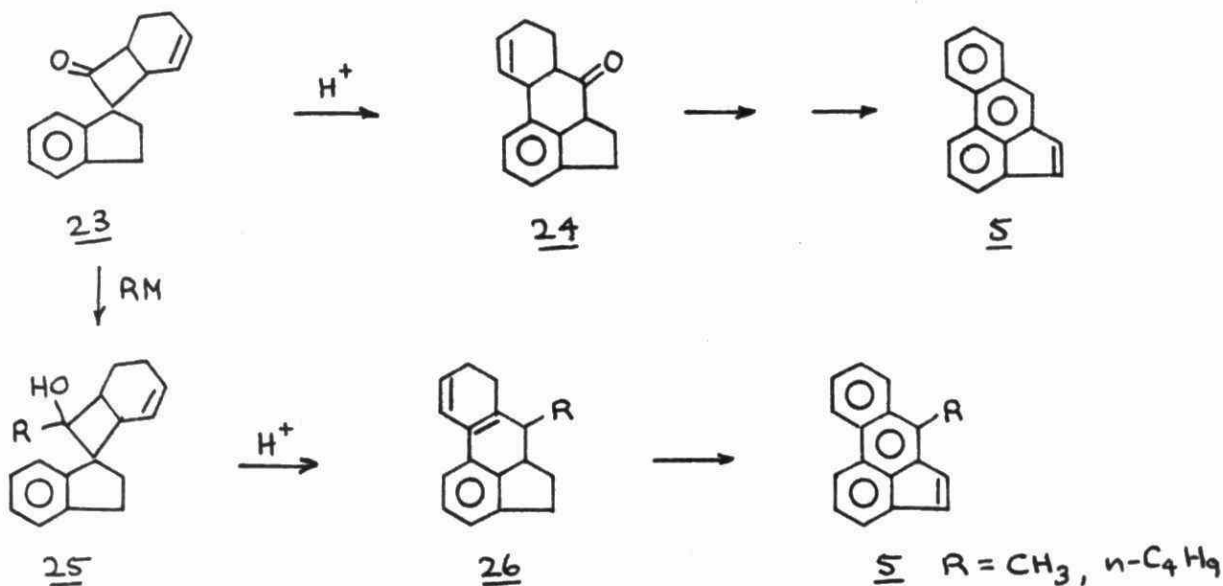
The parent derivative $\underline{5}$ was prepared by acid-rearrangement of $\underline{23}$ to $\underline{24}$. Reduc-

tion (LAH) followed by dehydration and dehydrogenation gave the parent $\underline{5}$ in

25% overall yield. The methyl and n-butyl derivatives of $\underline{5}$ were prepared by

alkylation of $\underline{23}$ (CH_3Li or n-C_4H_9Li), to $\underline{25}$, acid-catalyzed rearrangement to the

tetrahydroderivative $\underline{26}$ followed by dehydrogenation to $\underline{5}$. Overall yields of

$\underline{5}$ ranged from 20 to 30%. All of these compounds are currently being tested

for their mutagenic activity[9] and presence in air-particulate samples. Further

work in this area is being carried out to extend such syntheses in the prepara-

tion of oxygen and sulphur heterocycles as shown below.

X = O, S
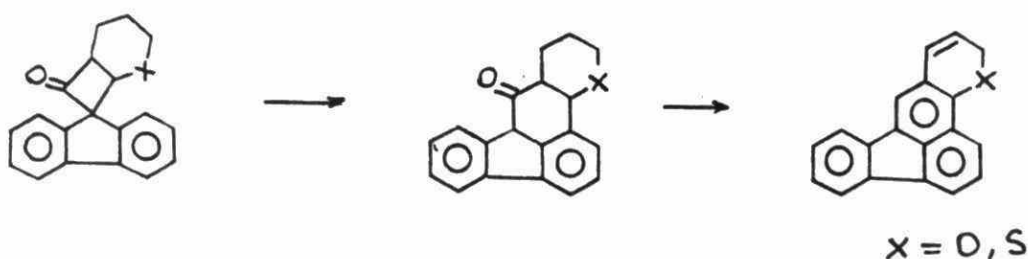
An example of the above synthesis has been achieved for an oxygen heterocycle[10].

References

1. H.V. Gelboin and P.O.P. Tso. "Polycylic Hydrocarbons and Cancer", Academic Press, N.Y. (1978).

2. E. Eisenstadt and A. Gold. Proc. Natl. Acad. Sci. USA 75, 1667 (1978).

3. A. Gold and E. Eisenstadt. Cancer Res. 40, 3940 (1980).

4. R.G. Harvey. Acc. Chem. Res. 14, 218 (1981).

5. E. Lee-Ruff, A.C. Hopkinson and L.H. Dao. Can. J. Chem. 59, 1675 (1981).

6. H. Lee and R.G. Harvey. J. Org. Chem. 47, 4364 (1982).

7. E. Lee-Ruff, H. Kruk and M. Katz. J. Org. Chem. 48, (in press) (1983).

8. J.K. Ray and R.G. Harvey. J. Org. Chem. 47, 3335 (1982).

9. M.F. Salomone and M. Katz. J. Nat. Canc. Inst. 66, 691 (1981).

10. E. Lee-Ruff and P. Duperrouzel. Can. J. Chem. 58, 51 (1980).

RETROSPECTIVE CORRELATION SPECTROSCOPY
AND ITS APPLICATIONS TO ATMOSPHERIC MONITORING

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ABSTRACT

A brief review of given of the concepts of ANALOGUE correlation spectroscopy in the context of atmospheric monitoring. It was proposed and developed in a number of laboratories in the 1960's. A number of commerical field correlation instruments have been developed for remotely sensing the presence and concentration of atmospheric contaminant species. These instruments use a unique correlation mask for each of the species, and thus sense one species at a time.

We have made a study of the potential of the related method: RETROSPECTIVE digital correlation spectroscopy for the concurrent sensing of a number of atmospheric contaminants , for each of which a unique digital correlation mask is specified, and convolved numerically with the digital field spectrum.

The results of this "proof of concept" study is described and the aims of the recently started research programme to apply the concept to atmospheric spectra is reviewed.

1: INTRODUCTION

Analogue field correlation spectrometers are commonly used for the monitoring and diagnosis of air quality in the vicinity of contaminant emission targets near the observing site.

The concept of ANALOGUE CORRELATION SPECTROSCOPY was proposed and developed by a number of workers in the 1960's (Barringer, 1964; Barringer and Shock, 1966; Bottema, Plummer and Strong, 1964a,b; Kay, 1967; Williams and Kolitz, 1968; Millan, Townsend and Davies, 1968; Millan, 1972; Millan and Hoff 1977). It has been incorporated into the design of a number of commercial instruments, principally by Barringer Research Ltd. of Rexdale, Ont.

In the analogue instruments, the field spectrum is repetitively compared with the simulated laboratory spectrum of one of the suspected molecular contaminants. The laboratory spectrum of the contaminant is simulated in a mask of alternate transparent and opaque slits. The transparent slits are arranged in a pattern which reproduces at the focal surface of the spectrometer, the pattern of strong spectral absorption features of the contaminant of interest. Correlation between the field spectrum and the mask (from which quantitative estimates of the concentration of contaminant can be inferred) is achieved by relative oscillation, at the focal surface, between the mask and the field spectrum. This is followed by phase-sensitive photo-electric detection of the resulting signal passing through the mask.

If $S_1(L)$ and $S_2(L-x)$ are the respective intensity distributions of spectrum (1), and spectrum (2) offset in wavelength L by x, then the cross-correlation or co-variance between them is:

$$C(x) = \int S_1(L) \, S_2(L-x) \, dx \qquad (1)$$

When applied to the analogue correlation spectrometer, $S_1(L)$ can be identified with the field spectrum and $S_2(L-x)$ can be identified with the offset and oscillating correlation mask. After suitable calibration of instrumental response, the real-time readout of the time averaged $C(x)$ can be interpreted in terms of number density or column density of the molecular atmospheric contaminant whose absorption spectrum is simulated by the correlation mask.

In the design and operation of these instruments, great attention has been paid to the optimization of the mask arrangement for each of the species (Millan and Hoff 1977). The analogue mask-correlation instruments are best suited to detection and monitoring of molecular species whose low resolution absorption spectra consist of a few unique well separated features, such as is trhe case for the SO_2 absorption bands between 300 and 310nm.

While the mechanical correlation mask can well represent low resolution spectra with separated and distinctive features, it is much less well suited to the representation of complex, multi-line, spectra, particularly at high resolution. Fabrication of individual correlation masks for such spectra poses almost insuperable mechanical difficulties. Another limitation of the analogue method is that a uniquely fabricated mask is needed for each species to be

sensed, and only one species can thus be sensed at a time with one instrument.

2:THE RETROSPECTIVE DIGITAL CORRELATION METHOD–PAST WORK

The principle of the correlation method is sufficiently powerful that it appeared to be worthwhile to investigate methods by which it can be applied concurrently to complicated, and possibly high resolution spectra of important species. We were encouraged to try this approach from the clear identification of NO_2 bands in the optical depth spectra of urban Toronto haze, particularly under conditions of "Brown Haze", even though our optical depth spectra are at relatively low resoluiton. (Nicholls, Peterson and Bunn, 1981)

Our spectra are recorded digitally and maintained in a digital database for more than 3000. It has been steadily augmented since 1977.

We therefore propsed to NSERC, with the support of Barringer Research Ltd. that it would be very worthwhile to investigate the potential of **RETROSPECTIVE CORRELATION SPECTROSCOPY** for the quantitative remote sensing of atmospheric contaminant species. This suggestion was accepted and we were awarded a P(roject) R(esearch) for A(pplication) to I(ndustry) grant ot make a numerical assessment of this method.

The detailed results of that study were given in the final report (Nicholls, 1982) and in a paper attached as an appendix to the report.(Cann and Nicholls, 1984)

In the retrospective digital method it is assumed that the field optical depth spectrum $S_1(L)$ is recorded and available in digital form. Then in principle, a digital numerical correlation mask $S_2(L-x)$ can be developed for each species of interest using the methods of numerical spectral synthesis. We have developed extensive software for this and applied it to a wide range of spectral diagnoses in laboratory, atmospheric, cometary and astrophysical circumstances from the far ultraviolet to the microwave region of the spectrum. An example of such work applied to long path absorption ballon spectroscopy of the stratosphere is described in the paper by Cann et al(1979).

A digital mask can be synthesized from knowledge of molecular constants, from which line locations, profiles, and intensities can be calculated. It can also be produced by digitizing laboratory spectra of the species in question.

After some preliminary empirical studies on our field optical depth spectra, we decided to adopt model numerical field synthetic spectra and model numerical correlation masks. We investigated how the resulting correlograms depended upon the character of the model spectra, model masks, resolution, "noise" content of the spectra etc. Details of this work will be given in the oral presentation of this paper. The results confirmed that in principle there is no reason why field spectra cannot meaningfully be assessed for presence of more than one contaminant species at a time. Whether this is done retrospectively (by bringing digital records of field spectra back to the laboratory computer) or whether it is done in situ , using a dedicated computer system while recording

field spectra will depend on circumstances. Both approaches are practical.

3: THE RETROSPECTIVE CORRELATION METHOD–FUTURE WORK

The results of this feasibility study were sufficiently encouraging that we proposed to the Ministry of the Environment an extension of the work oriented directly towards specific molecular species of importance in urban air quality assessment. The Ministry very recently approved the proposal and work has been in progress for a few weeks.

The proposed research programme has the following components:

1: Using the earlier study as a springboard, it is proposed to develop optimum digital correlation masks for spectral features of molecular species of importance to air quality considerations of urban and other atmospheres. Species such as NO, CO, NO_2, SO_2 are currently being studied.

2: Using methods of numerical spectral synthesis (Cann et al 1979), the long path atmospheric absorption signal (against daylight) will be realistically synthesized for a number of model atmospheres containing different column densities of molecular contaminants such as those listed above.

3: Retrospective correlation studies between the functions generated in (1) and (2) will be carried out to optimize spectral conditions (resolution, free spectral range, digital correlation masks etc.) for correlations with specific molecular species.

4: A programme of field observations will be proposed in the light of experience gained in tasks 1,2 and 3.

REFERENCES

BARRINGER, A.R.,(1964)
Proc. Third Symposium on Remote Sensing, (Univ. Mich. Press, Ann Arbor)

BARRINGER, A.R. and SHOCK, J.P.,(1964)
Proc. Fourth Symposium on Remote Sensing, (Univ. Mich. Press, Ann Arbor)

BOTTEMA, M., PLUMMER, W. and STRONG, J.H. (1964a)
Astrophysical Journal 139, 1021

BOTTEMA, M., PLUMMER, W. and STRONG, J.H. (1964b)
Astrophysical Journal, 140, 1640

CANN, M.W.P. and NICHOLLS, R.W. (1984)
Retrospective Correlation Spectroscopy In preparation for Applied Optics

CANN, M.W.P., NICHOLLS, R.W., EVANS, W.V.F., KOHL, J.L., KURUCZ,
  PARKINSON, W.H., and REEVES, E.M. (1979)
Applied Optics 18, 964

KAY, R.B. (1967)
Applied Optics 6, 766

MILLAN, M.M.(1972)
Ph.D.Thesis, University of Toronto

MILLAN, M.M., TOWNSEND, S.J., and DAVIES, J.H. (1969)
Report # 146, Institute for Aerospace Studies, University of Toronto.

MILLAN, M.M. and HOFF, R.M. (1977)
Applied Optics 16, 1609

NICHOLLS, R.W. (1982)
Final Report to NSERC, PRAI Grant P7908

NICHOLLS, R.W., PETERSON, R.N. and BUNN, F.E. (1981)
Applied Optics 20, 2767

STRONG, J.H. (1967)
Applied Optics, 6, 179

WILLIAMS, D.T., and KOLITZ, B.T. (1968)
Applied Optics, 7, 607

Monitoring Genotoxicity in the Environment Using Sister
Chromatid Exchanges in Mice *

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To be presented at: Technology Transfer Conference No.4
                     Ministry of the Environment, Ontario
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ABSTRACT:

Preliminary evidence in a study by B.N. Nayak suggested that sister chromatid exchanges (SCE) in both wild and inbred mice might provide us with a sensitive system for monitoring changes in environmental levels of genotoxic agents. This report describes attempts to increase the efficiency of the SCE test and some early results in testing the sensitivity of the system.

The SCE test involves differential staining of newly formed chromatids through the incorporation of 5-bromo-2'-deoxyuridine (BudR). BudR was introduced into mice by four routes and in several concentrations. Of these 80 mg/g body weight, serially injected intraperitoneally gave the most consistent results in both C3H (5.40±0.14) and wild mice maintained in the laboratory (5.56±0.12); BudR even in very high doses in drinking water resulted in poor differential staining; plain BudR tablets gave consistently high SCE values (9.07±0.58); and parafin coated tablets gave values which were low and consistent in C3H (4.18±0.10) but which fluctuated considerably in wild mice because in some cases very little BudR was absorbed. For subsequent work, serial injections were used although coated tablets may be more suitable if the quality of the coating process is improved.

For a monitoring system to be useable, a large number of samples must be examined which in this case means that it must be feasible to prepare a large number of slides efficiently. This was accomplished by reducing the handling of slides. After the cells were fixed on a slide, the slides were placed in a staining tray. Then the entire tray was immersed in Hoechst 33258 for 15 minutes, exposed to florescent lights while covered with Sorensen's pH 8.0 buffer and stained with Fisher's Giemsa. The slides were not handled individually until they were examined under a microscope. Besides permitting the staining of a large number of slides this technique also gave excellent differential patterns.

During the development of the above procedure the effects of two mutagens, mitomycin C (MMC) and methyl methanesulfonate (MMS) injected intraperitoneally into the mice, were examined. The MMC increased SCE levels up to threefold at 2 ug/g body weight and the MMS fourfold at 10 ug/g body weight. For a more natural introduction of mutagens, MMS was placed in the drinking water for 14 days. SCE values doubled with a total intake as low as 23 ug/g body weight of MMS and tripled with 90 ug/g body weight.

Thirty-two wild mice were also examined. These were collected from corn cribs in southwestern Ontario either before or shortly after the area was sprayed with pesticides. The two sets of samples differed significantly in SCE counts, 5.99±0.32 for the "prespraying" sample and 9.22±0.63 for the "postspraying" group. The latter also differed significantly from the baseline SCE values observed in laboratory mice (5.40±0.14).

Results obtained thus far continue to support the potential of the SCE technique for mutagen monitoring.

INTRODUCTION

Man through his efforts has either purposely or inadvertently released
into his environs a plethora of agents harmful to his health and that of
other organisms. Some of these agents are lethal, but many, especially at
low concentrations, are much more insidious in their action. In some cases
the latter may result in carcinomas years after the initial exposure, or in
mutations which, if recessive, may not be recognized for several generations
after they had occurred.(1-12) Needless to say there is a vital need for
effective tests to monitor agents which cause genetic damage, that is
genotoxins, in our environment. To date a number of tests have been
developed to determine whether specific agents are mutagenic and/or
carcinogenic.(13) Generally these tests have been routinely performed under
controlled laboratory conditions and have been applied to specific
carcinogenic and/or mutagenic agents.

This report presents some preliminary data on the feasibility of a
general test for genotoxicity that would involve organisms exposed to
possible genotoxins outside of the laboratory. That is a test that can be
done using natural populations of animals and/or plants. Such a test would
permit continuous monitoring of the environment by simply collecting samples
from populations already in place and could provide us with an EARLY WARNING
SYSTEM ON CHANGES IN GENOTOXIC LEVELS.

Among the tests which have been used to detect mutagenicity, there are
four that appear to have the above potential. These include those which are
involved in determining the incidence of chromosomal aberrations, dominant
lethals, micronuclei and sister chromatid exchanges (SCEs). There are also
several others which might be useful provided that sufficient genetic
information is available on the organisms which are to be used. These

include the detection of recessive mutations at specific loci and recessive
lethals using inversion chromosomes. Unfortunately most of these are of
limited value in studying genotoxicity in natural populations because either
they are relatively insensitive or a lengthy and labourious breeding program
is required. Only two appear to have much potential; these are the
micronucleus test(14,15) and the sister chromatid exchange (SCE)
test(16-19). Because Dr. B. Nayak (a former graduate student) and I had
already been looking at chromosomal abberations in wild house mice (Mus
musculus) for another purpose and because I and my students had been studying
populations involving this species for a number of years, we selected the
SCE test in this species for closer evaluation.

In deciding on the SCE test we were aware that many mutagenic and
carcinogenic agents increased SCE levels and also that there were some
exceptions.(16) Table 1 summarizes the results of a survey by Abe and
Sasaki (20) regarding the correlation between SCE inducers and
mutagenic/carcinogenic agents. They calculated the predictive value of the
SCE test for both mutagenicity and carcinogenicity to be about 80%. We also
knew that there has been considerable discussion on the nature of SCEs and
on exactly what it is that the SCE test is measuring. Such discussion is
irrelevent to our purposes as long as there is strong correlation between
SCE levels and the doses of mutagenic/carcinogenic agents to which the
organisms or tissues have been exposed.

In our approach we were concerned with:

1) modifying the technique used on laboratory mice to wild mice and
then to determine an SCE baseline level for inbred mice maintained in our
laboratory and to compare this to values obtained in freshly caught wild

mice;

    2) possible genetic differences between inbred and wild mice which would effect SCE levels;

    3) improving the consistency of the technique and making it less labour intensive so that more animals could be surveyed; and

    4) determining the exposure levels that would increase SCE levels significantly.

APPROACH USED

Mice from three inbred strains, C3H/J, C57B1/6J and DBA/2J were used to give us a baseline against which we considered all other animals. The wild mice came from corn cribs in southwestern Ontario. Corn crib populations were selected because large numbers of animals could be obtained when cribs were being emptied and because mice from different cribs would all have been exposed to the same basic diet and the same general environment. Heterogeneity in the environment related to farming practices and/or atmospheric fallout will of course occur.

To determine the effect of the environment in different parts of southwestern Ontario on inbred mice, C3H mice were housed in outdoor enclosures filled with corn. These were placed at various sites throughout the trapping area (Figure 1).

At the outset of this study, the mice were given intraperitoneally, 9 hourly injections of 40 ug/g 5-bromo-3'-deoxyuridine (BrdU) and 2 ug/g fluorodeoxyuridine. This was followed by 4 ug/g of colchicine 27 hours after the first BrdU injection. The mice were sacrificed 3 hours later. Femurs were

removed and the bone marrow cells were flushed into warm (37C) 0.075M KCl. The cells then went through five stages of fixation. After the slides were made, they were aged for 48 hours, then heated to 87-89C for 15 minutes in a phosphate buffer and stained in 5% Fisher's Giemsa. This is basically the Korenberg and Freedlender (21) technique.

INITIAL RESULTS

SCE values observed in the inbred strains studied, in wild caught mice maintained in the laboratory for 6 months, in wild caught mice injected with BrdU within 24 hours of capture and in C3H housed in cornfilled enclosures for four weeks are summarized in Table 2.

These results show that the three inbred strains gave very similar SCE values and that the wild mice housed under laboratory conditions for an extended period gave SCE values that fall well within the range of the inbreds. Therefore the genetic differences which occur between the inbred and the wild did not seem to have a major effect on SCEs. Newly caught mice gave significantly higher SCE values than inbreds or laboratory maintained wild mice. Similarly, the inbred mice housed outdoors showed a significant increase in SCE levels over those housed in the laboratory.

Table 3 gives more detailed results of the wild mice collected from different sites in southwestern Ontario. An analysis of the mean SCE/cell/mouse/site revealed a negative correlation between the SCE level and the distance to the nearest major industrial site (r= -0.79). The centres considered were the Detroit-Windsor complex, Sarnia and Toledo, Ohio.

REVISED PROCEDURE

The procedure used to obtain the early results gave slides of varying quality. Sometimes for no apparent reason the chromosomes were either condensed and poorly spread or no differential staining was seen. Furthermore, the procedure was very time consuming, especially the fixing and staining stages.

In the revised procedure 80 ug/g of BrdU were given intraperitoneally. The fixation phase was reduced to 90 minutes from over three hours. The slides were stained with Hoechst 33258 for 15 minutes in a concentration of 10 ug/ml of distilled water. The slides were then placed in a staining jar containing Sorensen's buffer at a pH of 8.0 and the containers, covered with saran wrap, were exposed to fluorescent lights for approximately 23 hours. At the end of this period a single slide was removed and stained with Fisher's Giemsa for 8 minutes. If no differential staining was seen the rest of the slides were exposed to the fluorescent lights for another ten minutes and another test slide was stained. This was repeated until good differential staining was observed and then all the remaining slides are stained. (We are grateful to Dr. Alena Krepinsky of York University for suggesting this staining procedure).

The revised procedure involves less effort because once the slides are made they are not handled individually until examination under a microscope. This then permits the preparation of a large number of slides at one time, and the test slides insure that no run is lost because of poor differential staining.

RESULTS WITH THE REVISED STAINING PROCEDURE

Table 2 also summarizes the results obtained with 80 ug/g body weight of

BrdU injected intraperitoneally and the new staining procedure. The baseline
count in C3H mice is higher then with 40 ug/g body weight BrdU, 5.63+0.14, as is
the SCE count of wild mice maintained under laboratory conditions for an extended
period (5.72+0.15). Freshly caught wild mice again gave values that were higher
than that seen in laboratory mice. In this case the wild mice could be
subdivided into two groups: PRE-PESTICIDE-SPRAYING SAMPLES and
POST-PESTICIDE-SPRAYING SAMPLES. The former gave SCE values only slightly higher
than the laboratory mice while the latter approached three times the baseline
value (Table 4).

Table 5 summarizes the results obtained in mice which had been exposed for
one week in the population enclosures. These mice unlike the earlier group
(Table 2) were injected with 80 ug/g body weight of BrdU. Of note is the fact
that mice after a only a single week of exposure showed very high SCE values at
some sites, almost three times the value seen in the laboratory mice.

FURTHER ATTEMPTS TO IMPROVE THE TECHNIQUE

Serial injection of BrdU into mice is time consuming and tedious, so we
considered several alternatives:

1) Addition of BrdU to drinking water for nine hours beginning at the
29th hour prior to sacrificing the animal. Since BrdU is light sensitive
the mice were kept in total darkness during this period. Doses ranging from
40 to 160 ug/g body weight were given. No differential staining was
observed.

2) Implantation of uncoated 50 mg BrdU tablets (obtained from Dr. J.
Heddle, York University). The mice were anesthetized with Avertin

(tribromoethanol), an anesthetic which totally inactivates an animal for about 20 minutes. The tablets were inserted subcutaneously between the scapulae. The SCE counts (Table 6) were considerably higher than in mice injected with 80 ug/g body weight/hour of BrdU. These results are consistent with those reported in the literature (22,23).

3) Implantation of paraffin-coated 50 mg BrdU tablets (obtained from Dr. R. Tice, Brookhaven Laboratory). The SCE levels (Table 6) were considerably lower than those observed with the plain tablets and somewhat lower than the values obtained with 80 ug/g BrdU injections. These levels would be suitable except that the amount of BrdU absorbed differed somewhat from mouse to mouse and at this point we do not know how much variability in SCE counts this caused. Paraffin-coated tablets will be tested further.

INTRODUCTION OF MUTAGENS

Mutagens can be introduced into an organism by injection, the respiratory system, the skin and the digestive tract. The normal laboratory procedure is injection. Much of the work, especially studies to determine the mutagenicity of a compound, has involved this route of introduction. This, however, is not the way compounds are taken in under natural conditions. We, therefore, began to compare the effects of intraperitoneal injection vs. oral intake of two compounds, methyl methanesulfonate (MMS) and mitomycin C (MMC). The objectives included determining the effects of: the time of exposure relative to BrdU treatment, the length of exposure for maximizing SCE induction and minimizing serious physiologic effects and different concentrations of a compound on SCE induction.

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The initial step was to inject these compounds at different times prior to and after the initial injection of BrdU. Table 7, based on four mice in each time period and for each compound, shows that for both of these compounds the SCE induction increased as they were introduced closer to the beginning of BrdU treatment. MMC continued to induce SCEs even after the injection of BudR, whereas for MMS the SCE level stabilized. Some preliminary studies also suggested that injections of MMS and MMC had little effect on SCE levels if given 48 hours before the initial injection of BrdU.

As a first step in introducing mutagens "naturally" into the mice we decided to explore intake via drinking water since the amount taken in is easily quantified. Some error is encountered but the error with food intake or skin absorption is as great if not greater. Table 8 gives the results of one of the experiments we have done showing the relationship between the uptake of MMS and SCE induction. The MMS in this case was given over a two week period. SCE levels increased with increase in MMS intake (r = 0.90).

The questions that we are trying to answer, for MMS and MMC, should be extended to other compounds, such as cyclophosphamide and 7,12 diethylbenzanthracene, and should also involve a comparative analysis.

SUMMARY OF RESULTS

1) The revised SCE procedure is giving good chromosomal preparations with excellent differential staining consistently.

2) The differences between SCE levels in laboratory maintained and either freshly caught wild mice or inbreds maintained under natural conditions indicate that the approach is sufficiently sensitive to detect changes in the environment.

A seven day exposure of C3H mice to a natural environment was found to be sufficient to alter the SCE count.

3) The differences between the laboratory maintained inbred mice and freshly caught mice are not primarily the result of genetic differences but rather variability in the environment to which these animals are exposed.

4) Preliminary evidence suggests that pesticides have an effect on SCE levels. This must of course be explored further.

5) There appears to be a correlation between SCE levels and distances to nearest large industrial complex.

6) Coated BrdU tablets may be a suitable replacement for serial injections but the coating of the BrdU tablets must be improved and further evaluation is required.

UPCOMING STUDIES

There are also several additional aspects which should be addressed in the near future:

1) Validation of the SCE results by using another procedure such as the MICRONUCLEUS TEST.

2) Examination of possible synergistic interactions between several mutagenic/carcinogenic agents characteristic of a particular environment.

3) Increasing the sensitivity of the SCE TEST for low concentrations of mutagenic agents perhaps through the use of DNA repair inhibitors such as hydroxyurea and aphidicolon. Some preliminary studies using these inhibitors in tissue culture cells have been reported.[24]

CONCLUSION

Results obtained thus far continue to support the proposal that the SISTER CHROMATID EXCHANGE TEST is an effective early warning monitor of environmental genotoxins.

REFERENCES

1. Fukino, H., S. Mimura, K. Inoue and Y. Yamane. 1982. Mutagenicity of airborne particles. Mutation Res. 102:237-247.

2. Lockard, J.M., J.W. Prater, C.J. Viau, H.G. Enoch and P.S. Sabharwal. 1982. Comparative study of the genotoxic properties of Eastern and Western U.S. shale oils, crude petroleum, and coal-derived oil. Mutation Res. 102:221-235.

3. Crossen, P.E., W.F. Morgan, J.J. Horan and J. Stewart. 1978. Cytogenetic studies of pesticide and herbicide sprayers. New Zealand Med. J. 88:192-195.

4. Behera, J. and S.P. Bhunya. 1980. In vivo effect of a commercial herbicide, Amiben, on bonemarrow and germ cells of mice. Caryologia 33:527-535.

5. Sobti, R.C., A. Krishan and C.D. Pfaffenberger. Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutation Res. 102:90-102.

6. Korte, C. and S.M. Jalal. 1982. 2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes. J. Heredity 73:224-226.

7. Moller, M. and I. Alfheim. 1983. Mutagenicity of air samples from various combustion sources. Mutation Res. 116:35-46.

8. Alink, G.M., H.A. Smit, J.J. van Houdt, J.R. Kolkman and J.S.M. Boleij. 1983. Mutagenic activity of airborne particles at non-industrial locations. Mutation Res. 116:21-34.

9. Nestmann, E.R., G.L. Lebel, D.T. Williams and D.J. Kowbel. 1979. Mutagenicity of organic extracts from Canadian drinking water in the Salmonella/Mammalian-Microsome assay. Environmental Mutagenesis 1:337-345.

10. Ohno, H., F. Hanaoka and M. Yamada. 1982. Inducibility of sister-chromatid exchanges by heavy-metal ions. Mutation Res. 104:141-145.

11. Stella, M., A. Montaldi, R. Rossi, G. Rossi and A.G. Levis. 1982. Clastogenic effects of chromium on human lymphocytes in vitro and in vivo. Mutation Res. 101:151-164.

12. Masaki-Paakknen, J., M. Sorsa and H. Vainio. 1981. Chromosome aberrations and sister chromatid exchanges in lead-exposed workers. Hereditas 94:269-275.

13. Anderson, D. 1978. An appraisal of the current state of mutagenicity testing. J. Soc. Cosmet. Chem. 29:207-233.

14. Heddle, J.A. and W.R. Bruce. 1977. On the use of multiple assays for mutagenicity, especially the micronucleus, Salmonella and sperm abnormality

assays. In Scott, D., B. Bridges and F.H. Sobels (eds) "Progress in Genetic Toxicology". Amsterdam: Elsevier.

15. Jenssen, D. 1982. The induction of micronuclei. Sandberg, A.V. (ed) "Sister Chromatid Exchange": 47-63. New York: Liss.

16. Latt, S.A., J. Allen, S.E. Bloom, A. Carrano, E. Falke, D. Kram, E. Schneider, R. Schreck, R. Tice, B. Whitfield and S. Wolff. 1981. Sister-chromatid exchanges: a report of the gene-tox program. Mutation Res. 87:17-62.

17. Latt, S.A., R.R. Schreck, K.S. Loveday and C.F. Shuler. 1979. In vitro and in vivo analysis of sister chromatid exchange. Pharmacologicl Reviews 30:501-535.

18. Block, A.W. 1982. Sister chromatid exchange methodology. Sandberg, A.V. (ed) "Sister Chromatid Exchange": 13-32. New York: Liss.

19. Knuutila, S. 1982. Sister chromatid exchanges in bone marrow cells. Sandberg, A.V. (ed) "Sister Chromatid Exchange": 165-173. New York: Liss.

20. Abe, S. and M. Sasaki. 1982. SCE as an index of mutagenesis and/or carcinogenesis. Sandberg, A.V. (ed) "Sister Chromatid Exchange":461-514. New York: Liss.

21. Kornberg, J.R. and E.F. Freedlender. 1974. Giemsa technique for the detection of sister chromatid exchanges. Chromosoma 48:355-362.

22. Allen, J.W., C.F. Shuler and S.A. Latt. 1978. Bromodeoxyuridine tablet methodology for in vivo studies of DNA synthesis. Somatic Cell Genet. 4:393-405.

23. King, M.-T., D. Wild, E. Gocke and K. Eckhardt. 1982. 5-bromodeoxyuridine tablets with improve depot effect for analysis in vivo of sister-chromatid exchanges in bone-marrow and spermatogonial cells. Mutation Res. 97:117-129.

24. Andersson, H.C. 1983. Hydroxyurea induces sister chromatid exchanges in G2: implications for the formation of chromosomal abberations. Hereditas 98:61-64.

TABLE 1. Correlation between mutagenicity and/or carcinogenicity of environmental chemicals and the SCE test.

MUTAGENICITY	CARCINOGENICITY	NO. OF CHEMICALS	SCE POSITIVE	SCE NEGATIVE
+	+	75	69	6
+	-	14	8	6
+	?	21	16	5
-	+	14	8	16
-	?	43	9	34
?	+	9	3	6
?	?	179	124	55
TOTAL		379	246	133

Based on: Abe and Sasaki (1982) SCE as an index of mutagenisis and/or carcinogenisis. In <u>Sister Chromatid Exchanges</u>: 461-514. A.A. Sandberg, ed. Alan R. Liss, Inc.

TABLE 2.   Mean SCE values in inbred and wild male mice maintained under various conditions and given either 40 ug/g body weight or 80 ug/g body weight of 5-bromo-3'-deoxyuridine (BrdU).

MICE	CONDITIONS UNDER WHICH THEY WERE MAINTAINED	40 ug/g/injection BrdU			80 ug/g/injection BrdU		
		NUMBER	MEAN SCE	$\pm$ S.E.M.*	NUMBER	MEAN SCE	$\pm$ S.E.M.*
C3H/J	Laboratory	32	3.39	0.07	18	5.63	0.14
C57Bl/6J	Laboratory	14	3.68	0.09	10	5.79	0.13
DBA/2J	Laboratory	9	3.92	0.13			
Wild	Laboratory for at least six months	18	3.52	0.06	9	5.72	0.15
Wild	Corn cribs - test was begun within 24 hours of capture	49	6.02	0.16	22	7.49	0.36
C3H/J	Enclosures in Essex County	14	5.62	0.14	12	10.27	0.34
C3H/J	Enclosures in Sarnia area	23	5.04	0.16			

*Standard error of the mean was calculated from the SCE values of individual mice.

TABLE 3: SCE values in wild mice collected from various locations is southwestern Ontario. Only samples with two or more mice are included.

LOCATION	NO. OF MICE	MEAN SCE/CELL ± S.E.M.*	KM FROM WINDSOR/DETROIT INDUSTRIAL COMPLEX
Fingal	2	5.37 ± 0.13**	154
Wardsville	4	5.36 ± 0.53	115
Ridgetown	12	5.58 ± 0.35	99
Tilbury	4	4.83 ± 0.19	58
Stoney Point	8	6.38 ± 0.13	48
Harrow	4	7.23 ± 0.28	28
Essex	5	6.95 ± 0.13	14
McGregor	8	6.27 ± 0.31	12

* Standard error of the mean for a locality was based on the SCE values of individual mice.

** The above mice were given 40 mg/g body weight of BrdU. The C3H control values were 3.39.

TABLE 4: SCE values observed in mice collected from corn cribs in southwestern Ontario before and after spraying was begun.

LOCALITY	DATE	NO. OF MICE	SCE/CELL	SEM/SAMPLE
PRESPRAYING:				
Trudell	4/26/83	4	5.84	0.66
Laramie	5/11/83	2	5.74	0.30
Sinasac	5/11/83	4	6.47	0.33
Rocheleau	5/26/83	2	5.60	1.61
Pooled:		$\overline{12}$	$\overline{5.99}$	$\overline{0.32}$
POST SPRAYING:				
Houle	6/25/83	6	7.66	0.21
Glendening	7/5/83	4	11.55	1.56
Pooled		$\overline{10}$	$\overline{9.22}$	$\overline{0.86}$

Prespraying and Postspraying results are significantly different (0.5% level).

TABLE 5: SCE values in C3H males placed in outdoor enclosures filled with corn and in appropriate controls maintained in the laboratory.

SITE	DISTANCE FROM AMHERSTBURG	ONE WEEK'S EXPOSURE
Delmore	3.8 km	10.50 ± 1.16
Paquette	5.8 km	7.30 ± 0.81
Laramie	8.1 km	9.35 ± 0.90
McKim	20.8 km	12.10 ± 1.23
Trudell	61.2 km	14.10 ± 1.76
Belanger	72.5 km	8.24 ± 1.03
Controls		5.89 ± 0.49

TABLE 6: A comparison of SCE counts in C3H and wild mice either injected with BrdU or implanted with BrdU tablets.

MICE	NO.	TREATMENT	SCE/CELL	S.E.M.
C3H males	12	80 ug/g/hr	5.40	0.14
C3H males	3	plain BrdU tablets* (50 mg)	9.06	0.58
C3H males	5	coated BrdU tablets** (50 mg)	4.18	0.10
Wild males (Rocheleau)	2	"	5.60	0.23

* Obtained from Dr. J. Heddle, York University.

** Obtained from Dr. R. Tice, Brookhaven Laboratory.

TABLE 7. SCE values in C3H mice that received either 2 ug/g body weight of mitomycin C or 10 ug/g of methyl methanesulfonate at various times before and after the first injection of BrdU. Both compounds were dissolved in Dulbecco's phosphate buffered saline. Four mice were used for each chemical and each time period.

TIME (hours)	SISTER CHROMATID EXCHANGES	
	MITOMYCIN C	METHYL METHANESULFONATE
Control (no compound)	3.99	
-26*	5.88	7.67
-13	5.90	13.99
- 6.5		12.65
- 3.25		14.26
- 0.75	6.85	13.70
+ 2	7.75	
+ 6.5	8.61	
+13	11.88	14.16
+24	6.40	

* This refers to twenty-six hours before the initial BrdU treatment. All mice were injected with 40 ug/g body weight of BrdU.

TABLE 8: SCE levels and amounts of methyl methanesulfonate (MMS) taken in
with drinking water by C3H mice. The SCE values are based on an average
of three mice per concentration except for the last where only one mouse
was available. The actual doses varied with water intake.

DOSE PLANNED (ug/g body weight)	AVE. WEIGHT OF MICE (g)	AVE. WEIGHT CHANGE (g)	TOTAL MMS INTAKE (mg)	SCE/CELL and SEM
0	31.1	+1.73	0	4.72 ± 0.02
2.5	25.4	+0.75	0.18	5.32 ± 0.23
10	28.4	+1.37	0.65	6.97 ± 0.85
40	28.6	+1.10	2.49	8.47 ± 2.68
160	26.6	+1.78	8.57	7.23 ± 0.06
640	28.4	-0.17	24.11	7.25 ± 0.24
2560	26.3	-4.80	72.01	12.25

FIGURE 1: Distribution of population enclosures in southwestern Ontario.

o   Population enclosures

⬤   Major industrial complexes
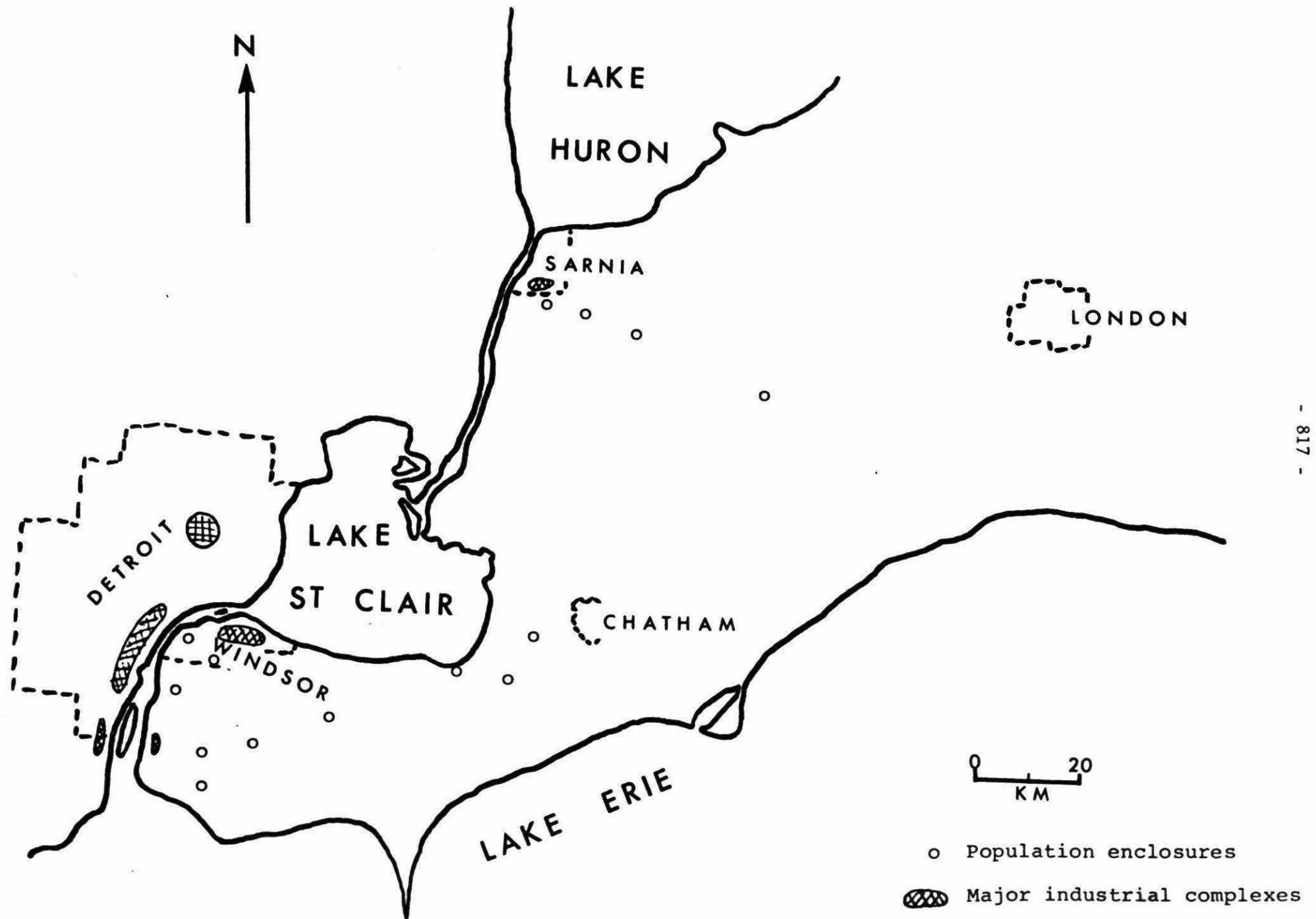
DEVELOPMENT OF A STRATEGY FOR PREDICTING
THE IMPACT OF ODOROUS POLLUTANTS FROM
FAST FOOD RESTAURANTS ON THE
SURROUNDING COMMUNITY

by

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ABSTRACT

It is still not possible to accurately assess whether citizens who complain about odors from fast food restaurants in their neighborhoods are truly offended by the emissions or are protesting against the litter and noise often created by patrons. Fast food operations do not normally affect the surrounding community over a wide enough area to generate sufficient odor complaints for statistical analyses.

Consequently, public attitude surveys have been carried out to determine the general reactions of individuals, selected at random in the neighborhood of a fast food outlet, to 25 commonly encountered odors. The results show that, in general, people like the odors of baking bread, barbeques and roses; but are disturbed by garbage, car/truck exhaust and fast food restaurant odors.

On the basis of the survey and documented complaints, a field sampling program was designed to collect samples for odor panel evaluations in the laboratory. A recently developed

Odor Impact Model, which will provide measures of detection, discrimination and complaint potential thresholds (as well as degrees of complaints), will facilitate the interpretation of laboratory generated data in terms of the number and frequency of complaints from the public reacting to a neighborhood odor source.

The Odor Impact Model is being applied to three odor sources. A domestic garbage landfill site and a sewage treatment plant provide facilities for convenient air sampling as well as good complaint documentation. The analysis of fast food outlets is handicapped by inadequate complaint data. The validated model can be used by industry and environmental regulatory agencies to characterize existing and proposed sources of odors with respect to their potentials for causing adverse community reactions.

A preliminary draft of a testing protocol for establishing the impact of an odorous source on the surrounding community is presented as a basis for future work.

INTRODUCTION

Of the various categories of air pollutants, odors are generally ranked as the major generators of public complaints to regulatory agencies in North American communities (1,2,3). Surveys of citizens living in the neighborhoods of odorous stationary sources indicate that odors can cause mental and physiological stresses on humans. Typical reactions are nausea, headache, loss of sleep, loss of appetite, impaired breathing and in some cases allergic reactions. However, objections against odors are generally raised, not because they are considered to be hazardous to human health, but because the human olfactory system is extremely sensitive to unpleasant stimuli (4).

Community odor complaints can be related to emissions from single or multiple fast food restaurants. Many of these convenience facilities appear to generate adverse reactions at considerable distances from their locations. In recent years these sources have received considerable attention from the public and regulatory agencies as more of them began to appear in the midst of residential areas and on the main streets of many North American cities (5). In spite of the alleged violations of air quality that communities have documented, it is still not possible to assess whether citizens who complain about odors from fast food restaurants in their neighborhoods are truly offended by the emissions or are protesting against the litter, traffic and noise normally associated with these operations. Although there are apparently widespread negative reactions to fast food restaurant odors, most communities do

not generate sufficient complaints to regulatory agencies for statistical analyses.

Reviews of technical literature (6) and government documents (7) as well as discussions with personnel from the Ontario Ministry of the Environment (8) indicated that there was a pressing need for the development of a technological basis for odor control in North America before effective odor compliance programs could be implemented. It has been emphasized that the technological needs include:

o procedures for establishing the validity of spontaneous odor complaints

o development and testing of improved odor sensory measurement techniques

o critical evaluation of atmospheric dispersion models for prediction of ambient odor levels

o development of relationships between ambient odor levels and annoyance thresholds for different communities

o development of procedures for locating and defining the alleged source or sources causing an odor problem in a community.

In order to meet its obligations to the citizens of Ontario, the Air Resources Branch of the Ontario Ministry of the Environment provided financial support to the Air Quality Group in the Department of Chemical Engineering at the University of Windsor, through the Research Grants Programme. The objective and scope of this investigation were defined during several discussions involving members of the Air Quality Group and the Air Resources Branch (9,10,11). It was apparent that lack of objective measurements of source and ambient odor levels and community responses to odor episodes handicapped the abilities of North American regulatory agencies to deal with odorous

stationary sources on more than a "nuisance" basis. Consequently, the Air Quality Group was expected to formulate guidelines which the Ontario Ministry of the Environment could use to develop codes regulating the sampling, emissions and controls associated with stationary odorous sources in general and fast food restaurants in particular.

In order to achieve this basic objective, a research programme was designed to facilitate:

o collection of representative odor samples from several fast food restaurants

o collection of ambient odor samples at various locations downwind from the facility at which source samples were being acquired

o correlation of ambient odor levels with source odor levels through refinements to the most appropriate atmospheric dispersion equation

o correlation of complaint potential thresholds and degrees of complaint of fast food restaurant odors determined under controlled laboratory conditions with citizen complaints actually registered with the Ontario Ministry of the Environment

o identification of any key odorant or odorants in the source emissions that could be responsible for community complaints.

PROGRAM DEVELOPMENT

A comprehensive examination of several fast food restaurants in the Windsor and Toronto areas showed that:

o there was a distinct lack of documented citizen complaints concerning fast food restaurants

o citizen responses could not be readily collected without creating unwarranted antagonism towards fast food restaurants in the community

o fast food restaurant emissions are too sporadic for routine ambient odor sample collection or real time instrumental monitoring of any key components.

In an effort to overcome these limitations a systematic search was conducted for odor sources where:

o citizen complaints to a regulatory agency are well documented

o emissions are relatively constant rather than sporadic

o emissions can be discussed in terms of readily identifiable major components

o existing odor control equipment operating parameters could be varied for assessment of odor removal efficiency and consequent changes in community responses.

During the past 4 years source and/or ambient odor sampling programs have been conducted at:

o an automotive foundry in Windsor, Ontario

o several fast food restaurants in Windsor and Toronto, Ontario

o a wastewater treatment facility in St. Catherines, Ontario

o a paint manufacturing facility in the Junction Triangle area in Toronto, Ontario

o a municipal solid waste landfill site in Avon Township, Michigan.

Automotive Foundry Studies

Preliminary evaluations of atmospheric dispersion models for predicting ambient odor levels were carried out during the summer of 1979 under the terms of an Ontario Ministry of the Environment Experience '79 grant (12). Ambient and source odor samples were collected simultaneously by 2 different teams. The source was an automotive foundry stack of 180 foot height with sampling ports installed at the 100 foot level. A newly developed in-stack dynamic dilution sampling head was used to dilute the stack gases with deodorized air to avoid condensation of water vapor and organic materials. Ambient

samples were collected at 2 or 3 locations downwind of the stack. Since the foundry was emitting a dark, readily visible and odorous plume, it was easy to define the locations where the highest ambient odor levels were to be expected.

Fast Food Restaurant Investigations

Prior to any on-site sample acquisitions typical fast food restaurant odors were generated in the laboratory by subjecting hamburger patties, onions and french fries, supplied by a major fast food chain, to the cooking techniques pertinent to that organization. Examination of the resulting odors with a portable infrared gas analyzer suggested that oleic acid could be used as a key odorant for relating sensory odor levels to instrumentally measurable concentrations. This concept of a key odorant was used for laboratory generated as well as source samples in the development of an odor dose-response relationship that expresses odor levels (as key odorant concentrations) in terms of their real life dimensions which have a direct impact on a community as complaint potential thresholds and degrees of complaint (13).

The frequency and duration of odorous assaults on the surrounding community were determined with human observers and a portable infrared gas analyzer while source sampling of fast food restaurants was carried out in Windsor and Toronto.

Wastewater Treatment Facility Program

The impact of the wastewater treatment plant on the surrounding community was assessed in terms of total odor and hydrogen sulfide as a key odorant. Impingement levels were

evaluated from measured source values through atmospheric dispersion modelling of steady-state emissions, using the traditional Gaussian plume dispersion relationship providing 1-hour averaging.

Paint Manufacturing Plant Studies

The source sampling program at a paint manufacturing establishment in the Junction Triangle area of Toronto indicated that a variety of technical and political problems were being created by the existence of multiple odor sources at a number of industrial sites. An intense community involvement attracted significant political attention.

Discussions with individuals familiar with the problems in this area showed that:

o public meetings were attended by Ontario Ministry of the Environment personnel as well as the local member of the Ontario Legislative Assembly

o area citizens as well as representatives of the Ministry of the Environment tracked offensive odors to sources at night and during daytime hours

o comprehensive ambient sampling/monitoring had been carried out by the Air Resources Branch mobile laboratory

o there was minimal or no documentation of the magnitudes of odor levels associated with any suspected source of odor emissions

o several industries had been issued with Ministerial Orders to improve their operations

o one industry had been charged with creating a neighborhood odor offence.

Solid Waste Landfill Site Studies

Validation of the Odor Impact Model can be a politically sensitive issue. Assessment of citizen reactions to any source

odor insults must be carried out without generating an abnormal awareness of any odor problems that have existed in the surrounding community for some time.

A scarcity of reliable Canadian complaint data for Odor Impact Model validation fostered a cooperative program with the Air Pollution Control Division of the Michigan Department of Natural Resources, the Environmental Protection Division of the Department of Attorney General, State of Michigan and the Engineering Department of the Charter Township of Avon, Michigan. A 770 unit mobile home park, located less than 500 feet from a domestic solid waste landfill site serving 14 Michigan townships, provided a convenient study area. The Avon Township Engineering and/or Fire Departments registered citizen complaints and provided validation of complaints whenever personnel were available to visit complainants usually within 15 minutes after telephone calls were completed.

Experiences with the Community Survey used by the Air Pollution Control Division of the Michigan Department of Natural Resources indicated that the 6 questions were of a highly leading nature. They tended to encourage participants to respond towards the formulation of a predetermined conclusion. Consequently, a relatively neutral Public Attitude Survey was developed and modified through a series of pre-tests with individuals having various vested interests. The final version has been tested only once in the neighborhood of a fast food outlet. The completion of the 32 questions required less than 5 minutes.

RESULTS AND DISCUSSION

Automotive Foundry Studies

The Hemeon (14) simplification of the Gaussian plume
dispersion equation, written in terms of source and ambient
dilution ratios, underpredicted the ambient odorous impacts
by factors of 100 to 200. Application of the Hogstrom (15)
Fluctuating Plume Model, which recognizes that short term peak
odor levels can be much higher than long term averages,
provided even poorer correlation of predicted and measured
impacts when detection thresholds were used as the basis of
nuisance prediction.

Even if the Hemeon and Hogstrom models had provided better
correlation of threshold data, it must be recognized that
detection threshold data are only useful for estimating the
atmospheric dispersion needed to make odorous source emissions
non-detectable. Dravnieks and O'Neill (16) have suggested that
in the neighborhood of a source where an odor is still perceived
after atmospheric dilution, odor intensity may be a more
important parameter than the odor detection threshold.
Consequently, the concept of intensity was introduced into
the Hogstrom Fluctuating Plume Model describing dispersion of
short term puffs. Source and ambient odor intensities were
evaluated according to the equal sensation function method using
250 ppm n-butanol as standard (12). Generally, the disagreement
between experimental and predicted values of ambient odor
intensity did not exceed a factor of 10.

Fast Food Restaurant Investigation

Figure 1 illustrates typical results of laboratory data defining an Odor Impact Model for fast food restaurant odors using oleic acid as the key odorous component. The Detection (fractional panel response) curve corresponds to panelists claiming to detect the presence of an odor because they actually smell something or because they are good guessors and can select the correct port delivering an odorous gas stream by chance. Since most panelists are not sure about the presence of an odor until they are exposed to much higher levels or concentrations, the concept of a Discrimination Threshold was introduced to account for the level at which panel members are absolutely sure that they can establish the presence of the odor under investigation. Depending on the quality (sour, burnt, fruity, acrid) and hedonics (pleasant or unpleasant) most people do not complain about an odor when they are first sure of its presence. The Complaint Profile or Annoyance Threshold has been defined as the level at which any fraction of odor panel members would be annoyed enough to complain when exposed to an odor for a specified period of time. The severity or degree of their complaints is measured in terms of the Annoyance Profile which is developed in an odor booth under controlled conditions using an arbitrary scale ranging from 0 to 10.

The relatively good agreement between the 50% panel responses for laboratory and source generated odors shown in Table 1 suggests that the use of oleic acid as a key odorant for fast food restaurant odors has considerable merit for future studies.
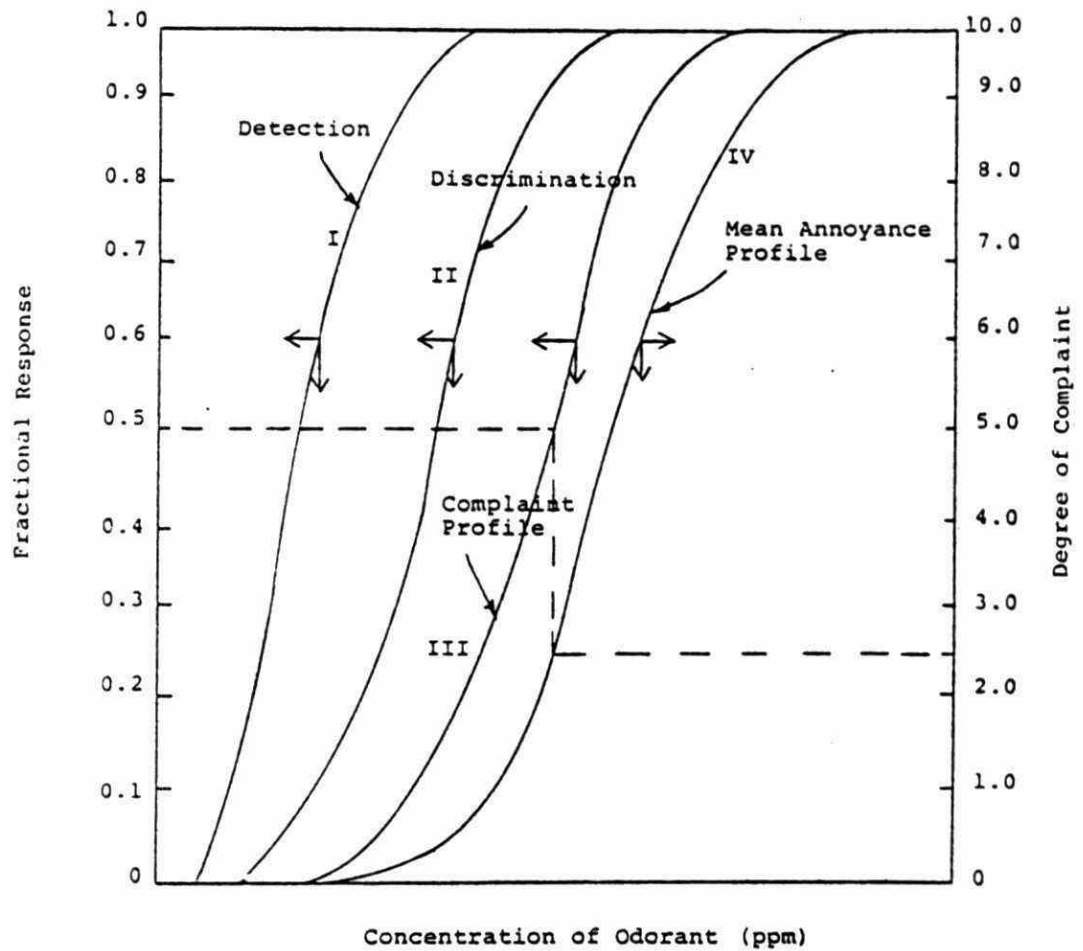
FIGURE 1: Illustration of Typical Odor Impact Model Profiles

TABLE 1:   Comparison of Odor Impact Model Threshold Data
(50% Panel Responses)

Odor Source	Laboratory Simulation	Fast Food Restaurant Exhaust	
Initial Oleic Acid Concentration	625 ppm	390 ppm	155 ppm
Threshold	Oleic Acid Concentration (ppm)		
Detection	3.9	5.5	3.9
Discrimination	7.7	11.2	7.8
Complaint Potential	8.4	11.3	7.8
(Degree of Complaint) 0-10 scale	1.6	2.2	1.8

Tracking of fast food restaurant odor emissions with human
observers and a portable infrared gas analyzer defined the
variability of impingement area locations and durations of
odorous perceptions illustrated in Figure 2.

Wastewater Treatment Facility Program

The wastewater treatment facility at St. Catherines, Ontario
provided a steady-state operation with essentially constant
emission rates of hydrogen sulfide, total reduced sulfurs and
sulfur dioxide which could be identified and collected at
various locations as key odorants.   The quality and hedonics
of these odorous components created a well documented citizen
complaint area which could be used to validate the results of
atmospheric dispersion modelling.

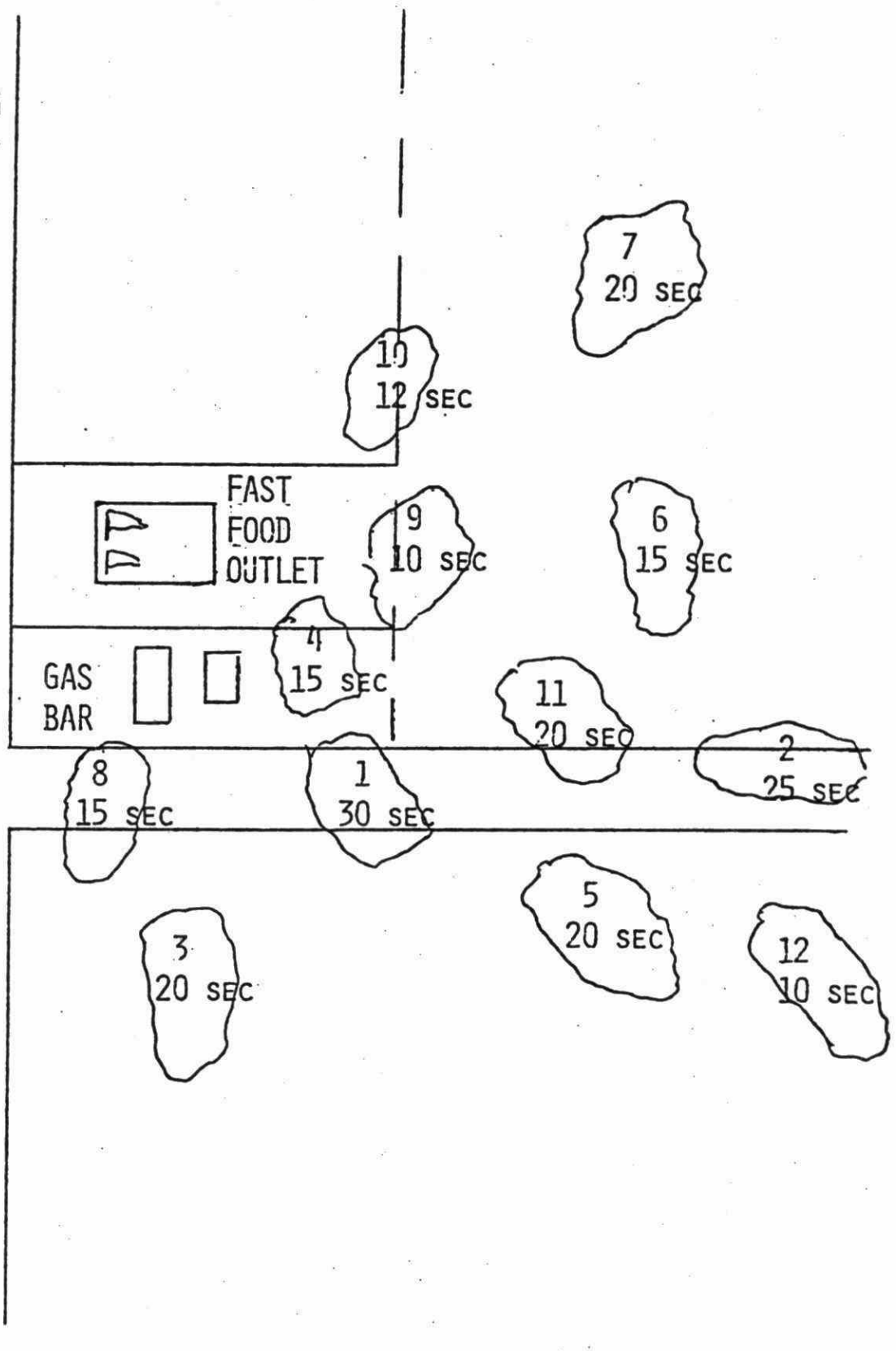Atmospheric dispersion of the steady-state emissions of

FIGURE 2: Typical Results of Tracking Fast Food
Restaurant Odor Emissions

hydrogen sulfide was modelled using traditional relationships providing 1-hour averaging. Since concentrations of hydrogen sulfide were established at the source, it was convenient to use the conventional form:

$$C_{(x,y,o)} = \frac{Q}{\pi u \sigma_y \sigma_z} \exp\left[\frac{-y^2}{2\sigma_y^2} - \frac{H^2}{2\sigma_z^2}\right]$$

to estimate downwind impingement concentrations of this odorant.

To facilitate the modelling of total odor dispersion it was useful to modify the standard dispersion equation to the form:

$$N_{(x,y,o)} = \frac{N_s V_o}{\pi u \sigma_y \sigma_z} \exp\left[\frac{-y^2}{2\sigma_y^2} - \frac{H^2}{2\sigma_z^2}\right]$$

The dispersion modelling showed that impingement concentrations of hydrogen sulfide could be high enough to generate adverse community reactions at distances of 875 feet and 1560 feet from the exhaust stack for C and D atmospheric stabilities, respectively. Essentially similar results were obtained for total odor dispersion.

The majority of documented citizen complaints originated from homes located 900 to 1800 feet from the wastewater treatment plant.

Paint Manufacturing Plant Studies

The conflicts in the Junction Triangle community made it difficult for the Air Quality Group to be accepted as an objective organization trying to establish background information. A personal tour of the area indicated that significant odor

problems were recognizable along the streets at sewer manholes. Apparently, discharges of scrubber liquors and perhaps even of products into the sewer system created unpleasant conditions in areas that were not necessarily downwind from suspected sources.

The paint manufacturing plant provided an opportunity for odor collection from 13 stacks. However, relation of source odor levels to citizen reactions and definition of complaint areas was hampered by the political climate in the community. Consequently, an arbitrary decision was made to define a "probable complaint area" in terms of ambient odor levels exceeding 1.0 odor units being sufficient evidence for "probable" complaint generation. Odor levels ranging between 0.5 and 1.0 odor units were assigned a rating of "possible odor impact". Predicted ambient odor levels below 0.5 odor units were projected to have minimal or zero effect on the surrounding community. Ambient impingement odor levels were calculated using appropriate modifications (in terms of source and ambient dilution ratios) of pertinent dispersion equations provided in Regulation 308 under the Environmental Protection Act (17).

Tables 2 and 3 illustrate the definition of areas of "probable" and "possible" odor complaints as determined by odor levels exceeding 1.0 odor units and ranging between 0.5 to 1.0 odor units, respectively. The 8 meter elevation odor levels are relevant for assessing the impact on neighboring 2-storey residences. It must be emphasized that the criteria used to define "probable" and "possible" complaint areas are

CONCENTRATION IN ODOR UNITS

DIST. FROM STACK	DISTANCE IN THE Y DIRECTION									
	0	10	20	30	40	50	60	70	80	90	100
10.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
40.0	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.000	0.000	0.000	0.000
50.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
60.0	0.000	0.000	0.000	0.927	0.012	0.000	0.000	0.000	0.000	0.000	0.000
70.0	0.000	2.673	1.826	1.190	0.044	0.002	0.000	0.000	0.000	0.000	0.000
80.0	1.054	2.190	1.662	1.157	0.097	0.007	0.001	0.003	0.000	0.000	0.000
90.0	0.991	1.858	1.627	1.132	0.140	0.016	0.004	0.009	0.001	0.000	0.000
100.0	0.913	1.620	1.553	1.084	0.220	0.028	0.012	0.020	0.003	0.000	0.000
110.0	0.837	1.442	1.462	1.028	0.270	0.043	0.023	0.033	0.008	0.000	0.000
120.0	0.771	1.304	1.366	0.972	0.310	0.061	0.036	0.046	0.014	0.001	0.000
130.0	0.716	1.192	1.274	0.910	0.338	0.081	0.050	0.058	0.021	0.002	0.000
140.0	0.671	1.100	1.180	0.869	0.359	0.101	0.064	0.068	0.029	0.004	0.000
150.0	0.634	1.023	1.110	0.825	0.373	0.121	0.077	0.076	0.036	0.007	0.000
160.0	0.604	0.957	1.040	0.786	0.383	0.141	0.089	0.083	0.043	0.010	0.000
170.0	0.580	0.900	0.978	0.751	0.390	0.159	0.100	0.088	0.040	0.013	0.000
180.0	0.559	0.851	0.923	0.720	0.395	0.175	0.109	0.092	0.053	0.016	0.000
190.0	0.542	0.807	0.873	0.692	0.398	0.190	0.118	0.095	0.056	0.020	0.000
200.0	0.526	0.760	0.829	0.667	0.401	0.204	0.126	0.097	0.060	0.023	0.000
210.0	0.512	0.733	0.790	0.644	0.403	0.216	0.134	0.099	0.062	0.026	0.000
220.0	0.499	0.701	0.754	0.623	0.404	0.227	0.141	0.102	0.064	0.029	0.000
230.0	0.487	0.673	0.721	0.604	0.404	0.236	0.147	0.104	0.066	0.031	0.000
240.0	0.475	0.646	0.691	0.587	0.404	0.245	0.154	0.106	0.068	0.034	0.000
250.0	0.464	0.621	0.664	0.570	0.403	0.252	0.159	0.108	0.069	0.036	0.000
260.0	0.453	0.599	0.639	0.555	0.402	0.258	0.165	0.111	0.071	0.038	0.000
270.0	0.443	0.577	0.615	0.540	0.400	0.264	0.170	0.114	0.073	0.040	0.000
280.0	0.433	0.557	0.593	0.526	0.397	0.268	0.175	0.116	0.074	0.042	0.000
290.0	0.423	0.539	0.572	0.513	0.394	0.272	0.180	0.119	0.076	0.044	0.000
300.0	0.414	0.521	0.553	0.499	0.390	0.275	0.184	0.122	0.078	0.045	0.000
310.0	0.405	0.504	0.535	0.487	0.387	0.277	0.188	0.125	0.080	0.047	0.000
320.0	0.395	0.488	0.517	0.474	0.382	0.279	0.192	0.128	0.082	0.049	0.000
330.0	0.387	0.473	0.501	0.463	0.378	0.280	0.195	0.131	0.084	0.051	0.000
340.0	0.370	0.459	0.486	0.451	0.373	0.280	0.197	0.133	0.087	0.052	0.000
350.0	0.369	0.445	0.471	0.440	0.368	0.280	0.200	0.136	0.089	0.054	0.000
360.0	0.361	0.432	0.457	0.429	0.362	0.280	0.202	0.139	0.091	0.056	0.000
370.0	0.353	0.420	0.443	0.419	0.357	0.279	0.203	0.141	0.093	0.058	0.000
380.0	0.345	0.408	0.431	0.406	0.351	0.277	0.205	0.143	0.095	0.060	0.000
390.0	0.337	0.397	0.418	0.398	0.345	0.276	0.206	0.145	0.097	0.062	0.000
400.0	0.330	0.386	0.407	0.389	0.339	0.274	0.206	0.147	0.099	0.064	0.000
410.0	0.323	0.375	0.395	0.379	0.334	0.271	0.206	0.148	0.101	0.065	0.000
420.0	0.316	0.365	0.385	0.370	0.328	0.269	0.206	0.150	0.103	0.067	0.000
430.0	0.309	0.356	0.374	0.361	0.322	0.266	0.205	0.151	0.105	0.069	0.000
440.0	0.302	0.347	0.364	0.353	0.316	0.263	0.206	0.152	0.106	0.071	0.000
450.0	0.296	0.338	0.355	0.344	0.310	0.260	0.205	0.153	0.108	0.072	0.000
460.0	0.289	0.329	0.345	0.336	0.304	0.257	0.204	0.153	0.109	0.074	0.000
470.0	0.283	0.321	0.337	0.328	0.298	0.254	0.203	0.154	0.111	0.076	0.000
480.0	0.277	0.313	0.328	0.321	0.293	0.250	0.202	0.154	0.112	0.077	0.000
490.0	0.271	0.305	0.320	0.313	0.287	0.247	0.201	0.154	0.113	0.078	0.000
500.0	0.266	0.298	0.312	0.306	0.281	0.244	0.199	0.154	0.114	0.080	0.000

☐ Region of Probable Odour Complaints   ☐ Possible Odour Complaints

TABLE 2: Odor Impingement Levels at an Eight Meter Height Resulting
From All Sources

DISTANCE ABOVE GROUND   0.0000000   METERS

CONCENTRATION IN ODOR UNITS

DIST. FROM STACK	DISTANCE IN THE Y DIRECTION										
	0	10	20	30	40	50	60	70	80	90	100
10.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
40.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
60.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
70.0	0.000	0.165	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80.0	0.144	0.284	0.035	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90.0	0.230	0.374	0.004	0.016	0.001	0.000	0.000	0.000	0.000	0.000	0.000
100.0	0.310	0.405	0.162	0.044	0.006	0.000	0.000	0.000	0.000	0.000	0.000
110.0	0.375	0.554	0.253	0.088	0.016	0.001	0.000	0.000	0.000	0.000	0.000
120.0	0.424	0.607	0.347	0.143	0.033	0.004	0.000	0.000	0.000	0.000	0.000
130.0	0.458	0.646	0.434	0.205	0.056	0.008	0.002	0.001	0.000	0.000	0.000
140.0	0.481	0.674	0.509	0.266	0.085	0.016	0.004	0.003	0.001	0.000	0.000
150.0	0.496	0.693	0.569	0.324	0.117	0.026	0.007	0.005	0.002	0.000	0.000
160.0	0.506	0.704	0.615	0.375	0.150	0.040	0.012	0.008	0.004	0.001	0.000
170.0	0.511	0.709	0.649	0.418	0.183	0.056	0.019	0.012	0.006	0.002	0.000
180.0	0.514	0.710	0.671	0.453	0.215	0.074	0.020	0.017	0.009	0.003	0.000
190.0	0.514	0.705	0.604	0.482	0.244	0.093	0.030	0.023	0.013	0.004	0.000
200.0	0.512	0.690	0.689	0.503	0.270	0.113	0.049	0.029	0.017	0.006	0.000
210.0	0.509	0.680	0.689	0.518	0.294	0.133	0.061	0.036	0.021	0.009	0.000
220.0	0.504	0.676	0.604	0.529	0.314	0.152	0.073	0.043	0.025	0.011	0.000
230.0	0.499	0.662	0.675	0.535	0.331	0.170	0.086	0.050	0.030	0.014	0.000
240.0	0.493	0.647	0.664	0.537	0.344	0.187	0.098	0.058	0.034	0.016	0.000
250.0	0.486	0.631	0.651	0.537	0.358	0.203	0.111	0.065	0.038	0.019	0.000
260.0	0.478	0.615	0.637	0.534	0.367	0.217	0.122	0.072	0.043	0.022	0.000
270.0	0.470	0.599	0.622	0.530	0.375	0.230	0.134	0.080	0.047	0.025	0.000
280.0	0.461	0.583	0.607	0.523	0.380	0.241	0.144	0.087	0.052	0.028	0.000
290.0	0.453	0.567	0.591	0.516	0.384	0.251	0.154	0.094	0.056	0.031	0.000
300.0	0.444	0.551	0.575	0.508	0.386	0.259	0.163	0.100	0.061	0.034	0.000
310.0	0.434	0.535	0.559	0.499	0.386	0.266	0.171	0.107	0.065	0.037	0.000
320.0	0.425	0.520	0.544	0.490	0.386	0.272	0.178	0.113	0.069	0.040	0.000
330.0	0.416	0.505	0.528	0.481	0.384	0.276	0.184	0.118	0.073	0.043	0.000
340.0	0.407	0.490	0.513	0.471	0.382	0.280	0.190	0.123	0.077	0.045	0.000
350.0	0.398	0.476	0.499	0.461	0.379	0.282	0.195	0.128	0.081	0.048	0.000
360.0	0.389	0.462	0.485	0.451	0.375	0.284	0.199	0.133	0.085	0.051	0.000
370.0	0.380	0.449	0.471	0.440	0.371	0.284	0.203	0.137	0.088	0.054	0.000
380.0	0.371	0.437	0.458	0.430	0.366	0.284	0.206	0.141	0.091	0.056	0.000
390.0	0.363	0.424	0.445	0.421	0.361	0.284	0.208	0.144	0.095	0.059	0.000
400.0	0.355	0.413	0.433	0.411	0.355	0.283	0.210	0.147	0.098	0.062	0.000
410.0	0.346	0.401	0.421	0.401	0.350	0.281	0.211	0.150	0.100	0.064	0.000
420.0	0.338	0.390	0.409	0.392	0.344	0.280	0.212	0.152	0.103	0.066	0.000
430.0	0.331	0.380	0.398	0.382	0.338	0.277	0.213	0.154	0.106	0.069	0.000
440.0	0.323	0.370	0.387	0.373	0.332	0.275	0.213	0.156	0.108	0.071	0.000
450.0	0.315	0.360	0.377	0.364	0.326	0.272	0.213	0.157	0.110	0.073	0.000
460.0	0.309	0.351	0.367	0.356	0.320	0.269	0.212	0.158	0.112	0.075	0.000
470.0	0.302	0.341	0.357	0.347	0.314	0.266	0.211	0.159	0.113	0.077	0.000
480.0	0.295	0.333	0.348	0.339	0.308	0.262	0.210	0.160	0.115	0.079	0.000
490.0	0.289	0.324	0.339	0.331	0.302	0.259	0.209	0.160	0.116	0.080	0.000
500.0	0.282	0.316	0.331	0.323	0.296	0.255	0.208	0.160	0.117	0.082	0.000

☐   Region of Possible Odour Complaints

TABLE 3: Ground Level Odor Impingement Resulting from all Sources

considerably more restrictive than those adopted by various
jurisdictions for odor control purposes. Normally,
residential zone odor levels are expected to be maintained
below 2.0 odor units through regulations or criteria/objective
standards (7,18).

It would be of considerable practical importance to relate
the results of the Air Resources Branch mobile laboratory
sampling/monitoring program to the "probable" and "possible"
complaint areas established during this investigation.

Solid Waste Landfill Site Studies

The location of the 770 unit mobile home park with respect
to the active landfill site is illustrated in Figure 3. Source
odor samples were collected:

o during dumping of freshly collected garbage

o during dumping of incinerated garbage

o 18 inches above ground level where odors were detected

o above a stagnant pool of water through which gases
percolated

o from a 2-inch observation well pipe sunk 30 feet
below the cap of a completed cell.

Acquisition of representative odor samples during the dumping
of garbage trucks or during sporadic gas evolution through
the cap on a completed cell still represents a technical
challenge. Reliable samples were collected above the stagnant
water pool and from the observation well for the development
of Odor Impact Models. Table 4 summarizes the Odor Impact
Model threshold data for the 2 representative landfill
samples.
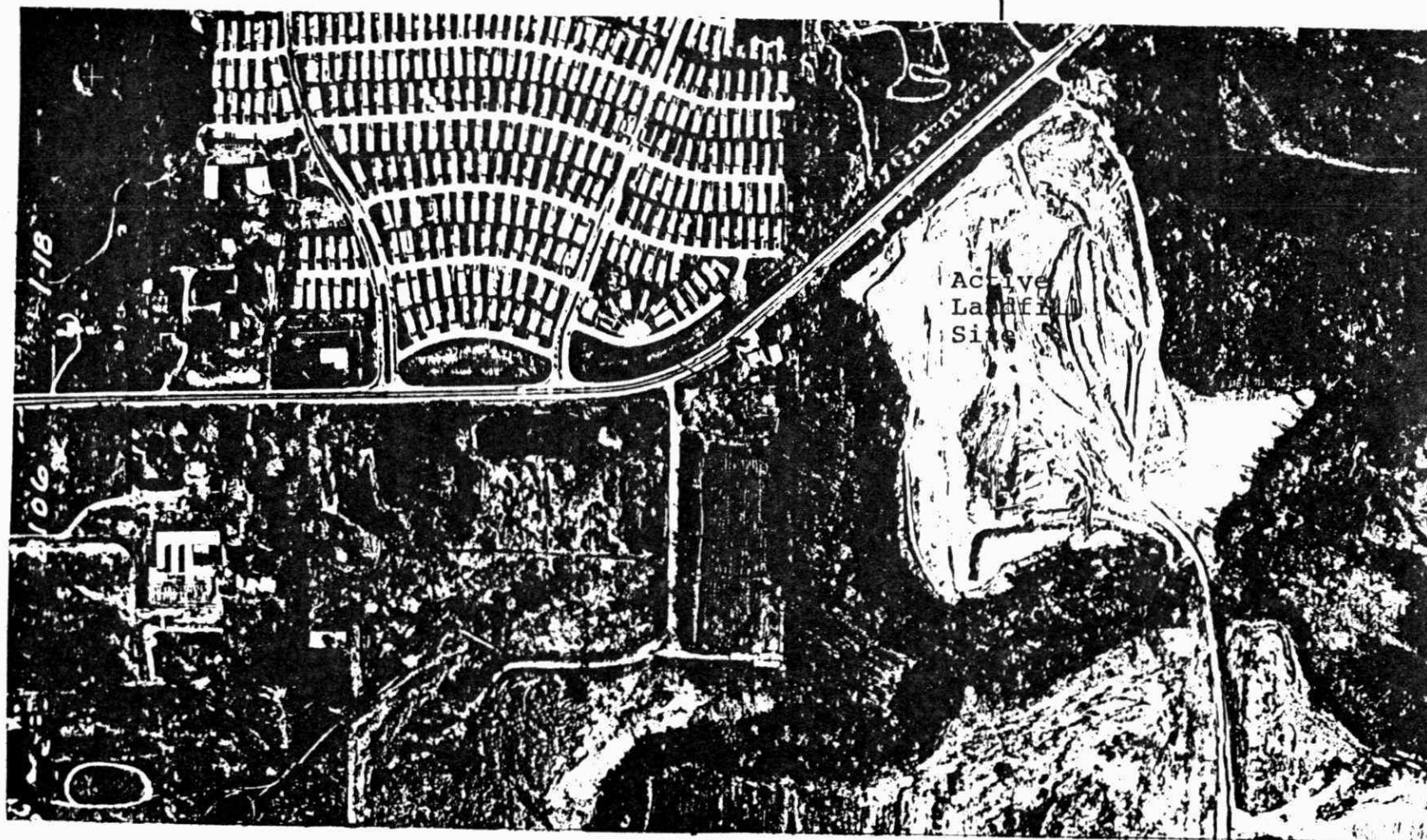
FIGURE 3: Relative Locations of Mobile Home Park and Active Landfill Site

TABLE 4: Summary of Landfill Odor Threshold Data
(50% Panel Responses)

Threshold (as odor units)	Odor Source	
	Water Pool	Observation Well
Detection	33	10,300
Discrimination	23	2,000
Complaint Potential	16	2,000
(Degree of Complaint) 0-10 scale	2.1	2.9

Tracking of odorous puffs across the top of the completed cells with 6 human observers indicated that under certain atmospheric conditions these puffs could move over considerable distances without losing their ability to create adverse receptor reactions.

Complaints from the mobile home park were most numerous during calm, humid evening or night time periods when apparent inversion conditions prevailed. Light winds from the southerly and easterly directions also generated 6 to 15 community reactions per day.

Figure 4 illustrates the Complaint Form used by the Township of Avon, Michigan to record citizen responses to the odors apparently transported from the landfill site. According

Std Distribution
due 10/19/83
mp

AVON TOWNSHIP CITIZEN COMPLAINT REPORT

COMPLAINANT _____Philipson_____ SEC#_____

ADDRESS _____140 Fontainbleau_____

DATE ____10-18-83____ TIME _1825_ P PHONE ____652-3826____

REPORT TAKEN BY:_____107_____ DEPT.____Fire____

NECESSARY TO CHECK: IMMED: __ WITHIN 24 HRS. __ A.S.A.P. __XXX__

COMPLAINT ____Odor coming from the dump_____

_____

_____1852 hrs. 105 advised odor was detected at_____

_____time of investigation   SEOCIA advised_____

_____

_____

_____

ROUTING VERIFICATION BY: _____
                              NAME - DEPARTMENT

REPORT ROUTED TO:

__ DEPARTMENT OF PUBLIC SERVICE   __ ASSESSING DEPARTMENT

__ ENGINEERING DEPARTMENT   X SUPERVISOR'S OFFICE

__ BUILDING DEPARTMENT   __ FIRE DEPARTMENT

__ HOUSING & ZONING INSPECTOR   __ OAKLAND COUNTY ROAD COMMISSION

ATTENTION: E. BORDEN   __ OTHER (specify) _____

+ +

I HAVE ALWAYS HEARD THAT FOR EVERY CALL WE GET OF A COMPLAINT
THERE ARE AT LEAST 25 or 50 OTHERS WHO ARE MAKING STATEMENTS
LIKE "WHY DON'T THEY DO SOMETHING ABOUT THAT" BUT DON'T BOTHER
TO CALL. I LIKE TO FEEL WE'RE HELPING MANY MORE PEOPLE THAN
THE COMPLAINANT BY ADDRESSING THE CONCERNS BROUGHT TO OUR
ATTENTION. E. Borden

INSTRUCTIONS FOR USE:
        1) The person receiving the complaint fills out the
top portion -who, what, when, where, and signs it after "Report
Taken By".
        2) The form (both white and cream parts still intact)
is then forwarded to the Department Head of the complaint re-
corder. The Department Head checks the routing, signs after
"Routing Verification By".
        3) The white part is then sent to the Supervisor's
office and the cream (larger) part sent to the action department.

FIGURE 4: Avon Township Complaint Form

to the data presented in Figure 5, some residents in the south east corner of the mobile home park have registered as many as 40 complaints in one year. Although more than half of all complaints have been validated through independent investigations by members of the Engineering or Fire Departments, frequent complainers are viewed with suspicion by regulatory agencies and the courts. In order to get a more objective assessment of the community odor problem, the Air Pollution Control Division of the Michigan Department of Natural Resources interviewed 42 residents in the neighborhoods surrounding the landfill site. The results of this community survey are summarized in Figure 6.

The format of the Michigan Department of Natural Resources Community Survey has been criticized because no efforts are made to control bias. As a result, the Air Quality Group developed the 32 question form described as Figure 7. A series of pre-tests was used for modification of the questions to insure that the questionnaire de-emphasized interests in any specific odor.

On a scale where +10 indicated liking an odor very much and -10 indicated liking an odor not at all, the odors associated with baking bread, roses and outdoor barbeques each scored a value of 6.0. On the other hand, the odors of ammonia, car/truck fumes and garbage received -8.2, -9.1 and -9.6 average ratings respectively. The odors characterizing Chinese restaurants, fried chicken and hamburger establishments were assigned respective magnitudes of -0.7, -1.3 and -2.2.

The responses to Question 32 are important in terms of

FIGURE 5: Major Complaint Locations
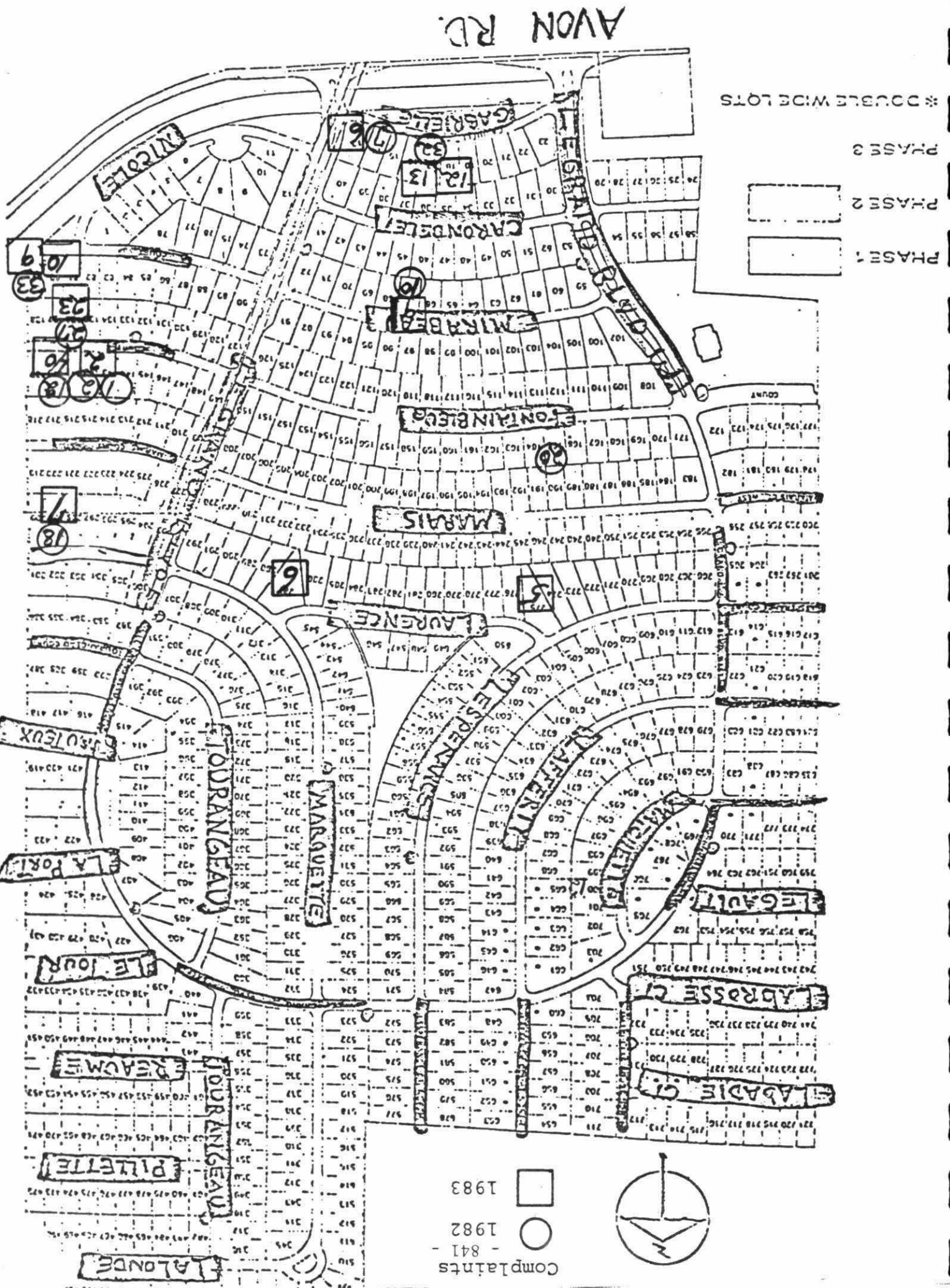
AIR POLLUTION CONTROL DIVISION
MICHIGAN DEPARTMENT OF NATURAL RESOURCES
COMMUNITY SURVEY

42 Residents Surveyed

1. DO YOU NOTICE ANY POLLUTION PROBLEMS AROUND YOUR NEIGHBORHOOD?
   yes 37 no 5 .

2. IF SO, COULD YOU PLEASE DESCRIBE THE TYPE OF POLLUTION?
   air 37 water - noise - aesthetic - other -

   description: Stench; Musty; Rotten Garbage; Landfill

   smell; Smells Like Someone Died.

3. CAN YOU IDENTIFY THE SOURCE OF THE POLLUTION? yes 37 no 5

   identification: Landfill site.

4. DOES THE POLLUTION APPEAR TO BE BOTHERSOME AT ANY PARTICULAR TIMES?

   description: Late at Night; When winds from South;

   In Evenings when Real Humid; Depends on Wind Direction

5. HOW DOES THE POLLUTION AFFECT YOU OR YOUR PROPERTY? Makes

   Stomach Turn; Cannot Relax Outside in the Evenings; Bothers

   Friends who Visit; Visitors Leave; Could Not Sell Home;

   Affects Property Values; Some Nights Must Close Windows.

6. WOULD YOU DESCRIBE THE IMPACT OF THE POLLUTION ON YOU OR YOUR PROPERTY?
   slight 6 moderate 11 very much 12 extreme 8 none -

   description: God Help Us; Real Nuisance; Mostly Early AM:

   Bad Between Midnight and Noon; When Wind from Southeast.

NAME _____

ADDRESS _____

CITY _____

NUMBER OF RESIDENTS IN THE LIVING UNIT: _____

DATE: _____ INTERVIEW BY _____

FIGURE 6: Summary of Community Survey

University of Windsor

DEPARTMENT OF CHEMICAL ENGINEERING

Environmental Study

1. Age _____

2. Sex (circle one)   M   F

3. Address   _____

     _____

4. Occupation   _____

5. Health (check one)   Excellent ☐
                           Good   ☐
                           Average   ☐
                           Fair   ☐
                           Poor   ☐

    If problems, please list: _____

    _____

    The following statements relate to various odours normally present in the air. Please read carefully and then rate each one individually based on your personal reactions. To complete the question circle the arrow (↑) which most closely matches how you feel about it.

Example: I like baseball.

     ↑   ↑   ↑   (↑)   ↑
    Not at all   O.K.   Very much

6. I like the smell of cut grass.

     ↑   ↑   ↑   ↑   ↑
    Not at all   O.K.   Very much

7. I like the smell of car and truck fumes.

     ↑   ↑   ↑   ↑   ↑
    Not at all   O.K.   Very much

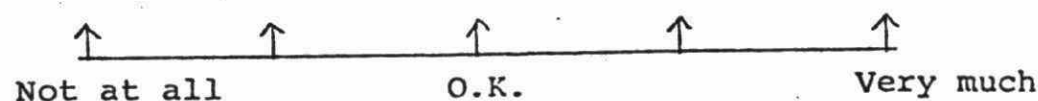FIGURE 7: University of Windsor Environmental Survey

8. I like the smell of fried chicken outlets.

Not at all   O.K.   Very much
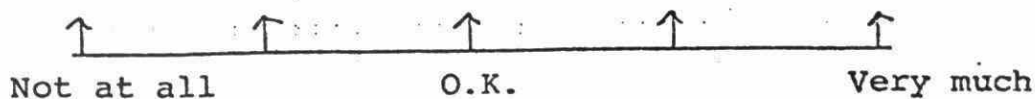
9. I like the smell from the sewers.

Not at all   O.K.   Very much
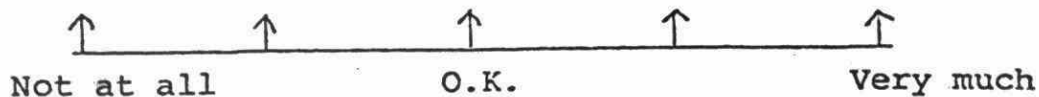
10. I like the smell of vinegar.

Not at all   O.K.   Very much
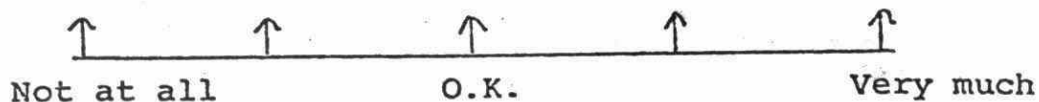
11. I like the smell of garbage.

Not at all   O.K.   Very much
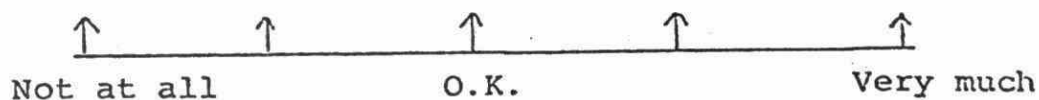
12. I like the smell of a hospital.

Not at all   O.K.   Very much
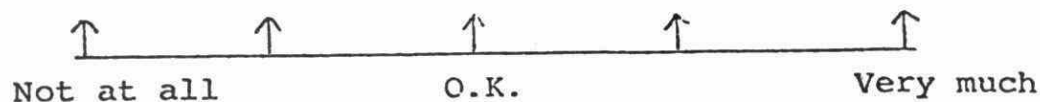
13. I like the smell at a Fruit Market.

Not at all   O.K.   Very much
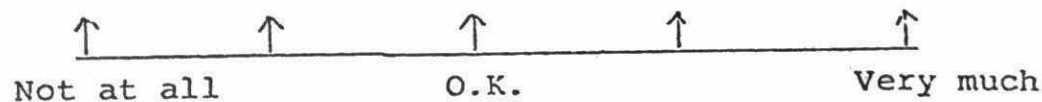
14. I like the smell of gasoline.

Not at all   O.K.   Very much
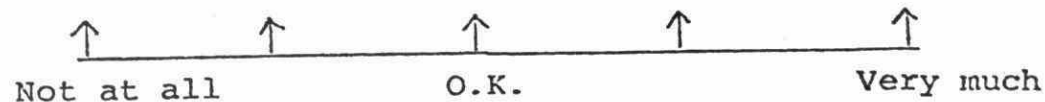
15. I like the smell of hamburger restaurants in the neighbourhood.

Not at all   O.K.   Very much
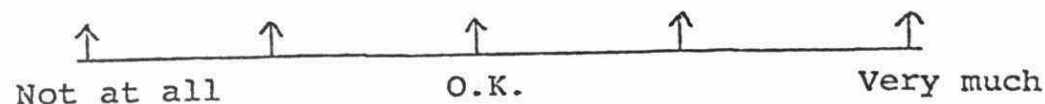
16. I like the smell of fresh popcorn.

Not at all   O.K.   Very much
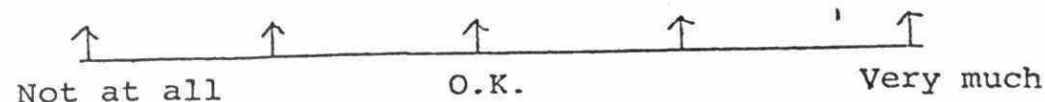
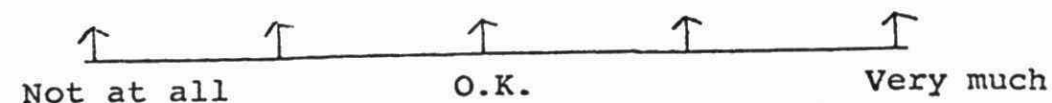17. I like the smell of ammonia.

Not at all   O.K.   Very much
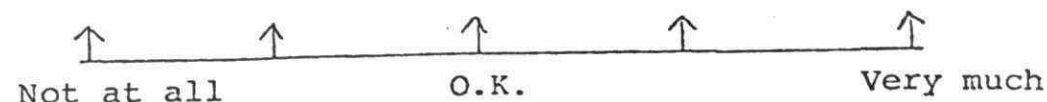
FIGURE 7: University of Windsor Environmental Survey (continued)

18. I like the smell of paint.

Not at all   O.K.   Very much
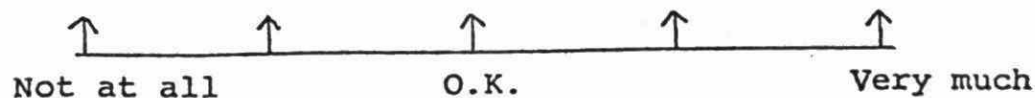
19. I like the smell of roses.

Not at all   O.K.   Very much
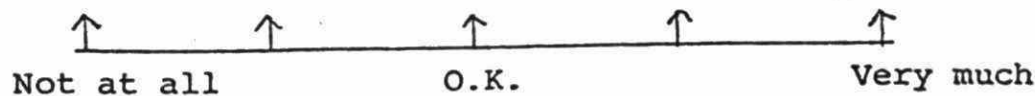
20. I like the smell of beer.

Not at all   O.K.   Very much
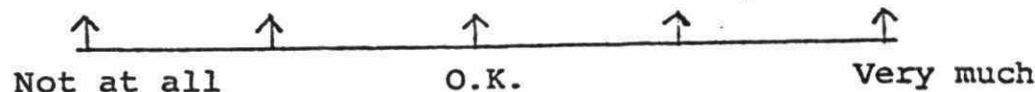
21. I like the smell outside a Chinese restaurant.

Not at all   O.K.   Very much

22. I like the smell of a locker/dressing room.

Not at all   O.K.   Very much
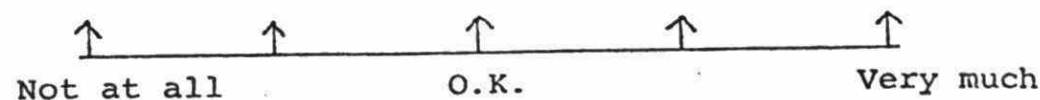
23. I like the smell of baking bread.

Not at all   O.K.   Very much
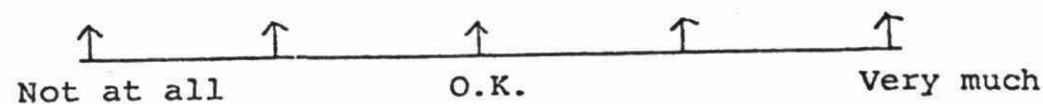
24. I like the smell of a wood fire.

Not at all   O.K.   Very much
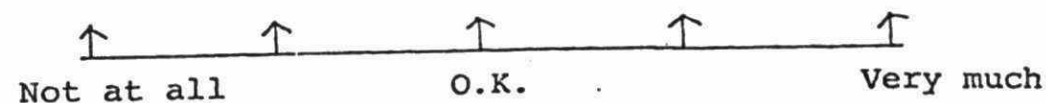
25. I like the smell of chocolate.

Not at all   O.K.   Very much
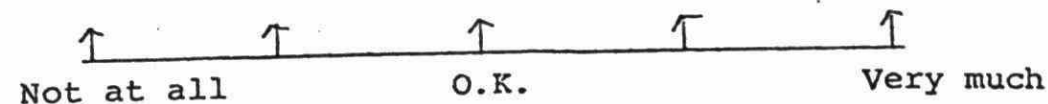
26. I like the smell of cigarettes.

Not at all   O.K.   Very much
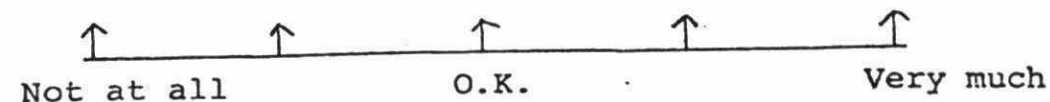
27. I like the smell of a carnival.

Not at all   O.K.   Very much

FIGURE 7: University of Windsor Environmental Survey (continued)

28. I like the smell of peanuts.

        Not at all   O.K.   Very much
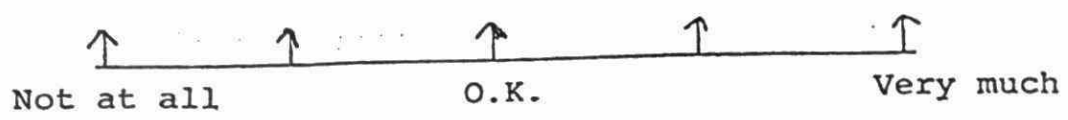
29. I like the smell of a barbeque.

        Not at all   O.K.   Very much
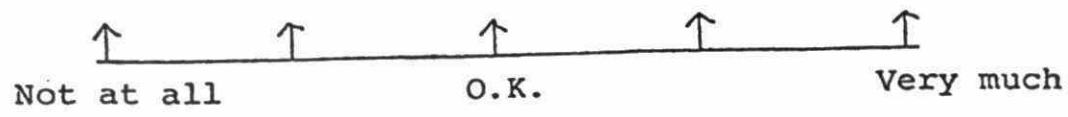
30. I like the smell of a leather jacket.

        Not at all   O.K.   Very much
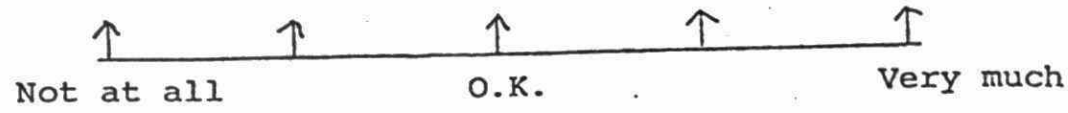
31. What smells in the air seriously bother you? Please list:

_____

_____

_____

_____

_____

32. This last question requires that you rank (in order of preference) 5 odors. Write the number which indicates your preference, below each odour.

1 = I like it best

5 = I like it the least

Example: football   baseball   hockey   soccer   basketball
             (3)   (2)   (1)   (4)   (5)

Please rank:

Hay   Swimming Pool   Barbeque   Hamburger Joint   Garbage

Many thanks for your cooperation.

FIGURE 7: University of Windsor Environmental Survey (continued)

potential control of fast food restaurant odors with hypochlorite scrubbing solutions. Improper operation of proposed wet scrubbers could lead to more serious odor problems if the original fast food odors are replaced with the smell of chlorine. Preliminary results showed that the chlorine odor of a swimming pool is less acceptable than the odors associated with hamburger outlets.

CONCLUSIONS AND RECOMMENDATIONS

The ultimate objective of this investigation was to initiate the development of a scientifically and legally acceptable procedure for assessing the impact of odorous pollutants from stationary sources on the surrounding community. On the basis of studies at 5 different types of stationary sources, it is clearly evident that several technical deficiencies still exist with respect to the development of odor control regulations in North America.

Before the use of syringes and scentometers can be eliminated completely, basic criteria must be established for dynamic olfactometers that are to be used for evaluating source and ambient odor levels.

Uncertainties in odor detection thresholds can be minimized through application of probability models that account for the possibility of correct responses from panelists without true discrimination [19,20].

It is clearly evident that the application of atmospheric dispersion models to the prediction of ambient odor levels is still highly speculative. The Hogstrom Puff Dispersion

Model (15) provides a fundamental starting point in terms of
dilution to threshold values or even more realistically in
terms of odor intensities.

The early attempts by Dravnieks and O'Neill (16) and
Lindvall and Radford (21) to relate ambient odor levels to
annoyance thresholds require extension through the validation
of the recently developed Odor Impact Model (13).

Recent experiences with legal problems involving odorous
stationary sources (4,22) indicate that in order to verify
that a suspected source (or sources) is or is not responsible
for alleged odor problems in a community, it is necessary to
prove that a scientifically and legally valid protocol has
been followed with respect to data acquisition.

The protocol proposed on the basis of this investigation
specifies measurements to be made at three levels involving
the:

o   source(s)

o   ambient air

o   affected population.

On-Site Measurements

In order to characterize the nature of any source emissions
experimental programs will:

o   identify the different locations where odors are
    released

o   determine the frequency and duration of odorous
    emissions at each location

o   determine the quantities (volumes) of typical odorous
    releases at each location

o   establish the odor levels in typical releases at
    each location in terms of dilution to detection

thresholds, intensities or concentrations of key
odorants

o identify, whenever possible, any key odorants that
could be responsible for community complaints through
infrared spectrometry and/or gas chromatography/mass
spectrometry.

Off-Site Measurements

In order to establish the magnitude of a community odor

problem it will be important to:

o identify the locations where odors have been and are
perceived

o determine the frequency and duration of perceived
odorous impacts

o measure the odor levels at the various locations where
odors have been perceived (in terms of dilutions to
detection thresholds, intensities or concentrations
of key odorants)

o identify, whenever possible, any key odorants that can
be related to perceived odors

Receptor Responses

Community reactions to existing ambient odors must be

characterized through valid odor dosage-response correlations

for a variety of conditions. This phase of the protocol would

involve:

o analysis of past and current odor complaints where
available

o analysis of the demographic nature of the affected
community (age distributions, socio-economic activities
and political forces are important)

o analysis of answers to an appropriate odor-survey
questionnaire conducted in the affected community and
a matching control area (to determine citizen prejudices
and individual reactions to perceived odors)

o development of procedures for establishing the validity
of current complaints

o development of an odor indexing or Odor Impact Model that will relate community odor detection, discrimination and complaint potential thresholds, as well as degrees of complaint, to the odor levels under consideration.

Successful verification that a suspected source (or sources) is or is not responsible for alleged odor problems in a community will require correlation of the data from on-site, off-site and receptor response studies.

Simultaneous sampling of source and ambient odors at times when complaints are being registered would help to settle controversial issues.

Fingerprinting, through identification of key odorants at on-site and off-site locations would, when coupled with reliable atmospheric dispersion modelling techniques, provide convincing technical evidence that a specific source is or is not responsible for community annoyance. Successful characterization of odor transport from source to receptors would require acquisition of:

o topographical data including locations of any wind screens

o meteorological factors including wind speed and direction as well as frequencies of wind directions

o ambient air parameters including temperatures, humidities and cloud covers

o locations of alternate odor sources such as sewer manholes, garbage bins and/or mobile sources.

Continued studies are still required if North American regulatory agencies are to develop legislation pertaining to odorous emissions. Lack of objective techniques for the measurement of source and ambient odor levels and community responses to odor episodes prevent control agencies from considering odorous industrial discharges to be more than "nuisance" problems.

NOMENCLATURE

$C_{(x,y,o)}$ = 1-hour average concentration at ground level point of impingement ($\mu g/m^3$)

Q = source emission rate of pollutant (g/s)

$N_{(x,y,o)}$ = C/C_{50} (dimensionless)

N_s = C_{source}/C_{50} (dimensionless)

C_{50} = 50% detection threshold concentration (ppm)

C_{source} = odor concentration at source (ppm)

C = downwind odor concentration (ppm)

u = wind velocity (m/s)

σ_y = crosswind plume standard deviation (m)

σ_z = vertical plume standard deviation (m)

H = effective plume height (m)

V_o = volumetric flow rate at source (m^3/s)

REFERENCES

1. Odors from Stationary and Mobile Sources, Board on
    Toxicology and Environmental Hazards, Assembly of Life
    Sciences, National Research Council, National Academy
    of Sciences, Washington, D.C. (1979).

2. Provincial Ministries and Departments of the Environment,
    personal communications with directors and local unit
    managers across Canada (1976-1983).

3. Reith, H.F., Resource Specialist Air Quality Division,
    Department of Natural Resources, State of Michigan,
    Pontiac, Michigan, personal communications (July 1983).

4. Environmental Hearing, Avon Township, Michigan vs
    Southeastern Oakland County Incinerator Authority, (October
    19, 1983).

5. Weller, B., Director of Engineering, McDonald's Restaurants,
    Oakbrook, Illinois, personal communications (April 1979).

6. Prokop, W.H., "Developing Odor Control Regulations:
    Guidelines and Considerations", JAPCA 28, 9-22, (1978).

7. Regulatory Options for the Control of Odors, A Report to
    the Congress of the United States; Strategies and Air
    Standards Division, Office of Air Quality Planning and
    Standards, US EPA, Research Triangle Park, N.C., 27711 (1979).

8. Martin, C., Emission Technology and Regulation Supervisor,
    Air Resources Branch, Ontario Ministry of the Environment,
    personal communication (1979).

9. Ozvacic, V., Head, Source Measurement Unit, Emission
    Technology and Regulation Development Section, Environmental
    Planning Division, Ministry of the Environment, 880 Bay
    Street, Toronto, Ontario, M5S 1Z8, personal communications
    (1979-83).

10. Suboch, W.P., Head, Industrial Organic Unit, Quality
    Protection Service, Ministry of the Environment,
    1 St. Clair Ave. West, Toronto, Ontario, M4V 1K6, personal
    communications (1979-83).

11. Barrow, E.T., Head, Control and Process Technology Unit,
    Emission Technology and Regulation Development Section,
    Environmental Planning Division, Ministry of the
    Environment, 880 Bay Street, Toronto, Ontario, M5S 1Z8,
    personal communications (1979-83).

*12. Viswanathan, S., R. Del Farra, W. Keller, T. Harris,
    A.W. Gnyp, C.C. St. Pierre, G.P. Mathur, S.J.W. Price,
    D.S. Smith and R. Aldi, "Evaluation of Dispersion Models
    for Odor Nuisance Prediction - An Experience '79 Program
    Industrial Case Study", 72 pages (1980).

*13. Gnyp, A.W., E.M. Poostchi and C.C. St. Pierre, "Development of Procedures for Assessment and Documentation of Odorous Impacts on a Community", 176 pages (1982).

14. Hemeon, W.C.L., "Malodors - A Basis for Regulations", JAPCA 21, 770-773 (1971).

15. Hogstrom, U., "A Method for Predicting Odor Frequencies from a Point Source", Atmospheric Environment, 6, 103-121 (1972).

16. Dravnieks, A. and H.J. O'Neill, "Annoyance Potentials of Air Pollution Odors", American Industrial Hygiene Journal, 40, 85-95 (1974).

17. Environmental Protection Act, "Revised Statutes of Ontario, 1980", Chapter 141, as amended 1981, Chapter 49 and "Regulation 308 (General-Air Pollution, Revised Regulations of Ontario, 1980", Queen's Printer of Ontario (March 1982).

18. 1981-1982 APCA Directory and Resource Book. Special Anniversary Edition APCA 75 years, page 161. Air Pollution Control Association, Pittsburgh, PA 15230.

19. Viswanathan, S., G.P. Mathur, A.W. Gnyp and C.C. St. Pierre, "Application of Probability Models to Odor Threshold Determinations", ATMOSPHERIC ENVIRONMENT, 17, 139-143 (1983).

20. Poostchi, E., A.W. Gnyp and C.C. St. Pierre, "Comparison of Models Used for the Determination of Odor Thresholds", ATMOSPHERIC ENVIRONMENT. Recommended for publication after minor revisions.

21. Lindvall, T. and E.P. Radford, Eds., "Measurement of Annoyance Due to Exposure to Environmental Factors", A Report from the Fourth Karolinska Institute Symposium on Environmental Health, Env. Res., 6, 1, (1973).

22. Province of Ontario Provincial Offences Courts. "Regina vs S.C.M. (Canada) Ltd.", (November 29-30, 1982).

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*Final Reports Submitted to Ontario Ministry of the Environment

CHEMICAL SPECIATION OF AIRBORNE PARTICULATES

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ABSTRACT:

Although neutron activation analysis provides high sensitivity and a low analytical blank, it does not allow determination of the chemical state of the elements detected. A method has been proposed whereby organic vapours are used to form volatile complexes of inorganic constituents of airborne particulates. These complexes could then be volatilised, removed from the sample and collected for neutron activation analysis. If the complex formation and volatilisation occur at different temperatures for different compounds of the same element, chemical speciation of that element should be possible.

The reactions of acetylacetone and its fluorinated derivatives with compounds of cadmium, iron and chromium are being studied to assess their possible utility for speciation. It has been found that although cadmium and its compounds react with the ligands tested, these reactions are too slow to be useful in analysis. The reactions of iron and chromium, however, are more rapid. The experimental methods adopted for this work will be described , results obtained to date will be discussed, and the course of future research on this topic will be outlined.

In order to prepare for future field studies in speciation, research in neutron activation analysis is also being carried out. A new method for the analysis of gamma-ray spectra has been developed and compared with other methods previously reported in the literature. The method developed in this laboratory proved to be as accurate as most other methods and more reliable in peak detec-

tion and in the treatment of multiplet peaks than several previously proposed techniques.

A new approach to optimisation of analytical procedures for activation analysis is under study. This new method, simplex optimisation by advance prediction (S.O.A.P.), involves calculation of spectra expected under given conditions and application of this information to simplex optimisation. The optimisation of single-element neutron activation analysis can now be achieved and multielement activation analysis is now being implemented . The progress made in these areas and the work yet to be done will be briefly described.

Introduction:

The proper assessment of the nature, behaviour and hazards of solid material present in the atmosphere requires methods for the determination of the composition of these materials. As airborne solids are found in low concentrations, highly sensitive techniques are needed for their analysis. These techniques should, ideally, provide data concerning not only the elemental composition of particulates but also the chemical forms of each element found.

Neutron activation analysis (NAA) is a highly sensitive analytical tool and has long been used for the elemental analysis of airborne particulates. NAA however, does not itself provide any information about the chemical form of the elements determined. This project addresses this problem.

It has been shown (1) that certain organic substances are capable of reacting with inorganic compounds to form volatile metal complexes. The approach to speciation adopted here employs this phenomenon to remove elements of interest from samples by volatilisation using organic vapours. The name reactive gas extraction has been given to this process.As different compounds may be expected to react with these vapours at different temperatures, selective volatilisation and hence speciation should be possible. At present, the chemistry of promising volatilisation reactions is being studied.

Once volatilisation of an element has been achieved, the volatile complex can be condensed and submitted to activation analysis. The use of only gaseous reagents should preserve the low analytical blank of NAA and the high sensitivity of NAA will permit the analysis of small quantities of material.

Experimental Methods:

Volatilisation experiments are carried out in a stream of carrier gas (nitrogen) mixed with ligand vapours. This gas stream is passed to a reaction tube which is held at a controlled temperature. The solid sample is placed in a pyrex cup provided with a thermocouple for temperature measurement. The gas, with volatilised complexes, is focussed by a capillary onto a collecting surface whose temperature is controlled to permit deposition of the complexes.

The progress of the volatilisation , in some experiments, will be monitored by means of radioactive samples and a radiation measurement instrument. It was found that a conventional tube furnace absorbed too much of the radiation. Accordingly, heating tapes are now used for temperature control. Separate temperature control is used for the ligand vapourisation, reaction, transfer and deposition zones. Figure 1. shows the experimental arrangement.

The ligands under study are moderately expensive. Therefore, to reduce losses, a cold trap and a peristaltic pump are used to recycle unreacted ligand. Figure 2. indicates the provisions for recycling.

Temperatures at the sample are measured by a digital thermocouple thermometer and recorded using a TRS-80 model I microcomputer. Radioactivity is monitored and recorded by a NaI(Tl) detector and a Canberra model 30 multichannel analyser operated in the multiscaling mode. The thermometer, multichannel analyser and the temperature program for the reaction zone of the volatilisation apparatus are controlled by a home-made electronic controller. Figure 3. gives the configuration used for data logging and temperature control.

It has been found that the volatile complexes and the ligands condense from the gas phase at similar temperatures. This makes collection of the complexes difficult and complicates radiotracer experiments. Reduction of the complexes to the metal and the free ligand using hydrogen has been reported for some complexes (2) and will be investigated as an alternative method for deposition of volatilised metals.

Ligands:

Several classes of compounds including β- diketones and dialkyldithiocarbamates have been employed for the creation of volatile metal complexes (3). The most important of these are the β- diketones and their fluorinated derivatives. The ligands investigated to date are 2,4-pentanedione, (AC) 1,1,1-trifluoro-2,4-pentanedione (TFA) and 1,1,1,5,5,5-hexafluoro-2,4,-pentanedione (HFA). These reagents have been purchased and used as received.

Results and Discussion:

Initial experiments concerned the volatilisation of cadmium. The volatilisation of cadmium and its compounds with the ligands listed above, however, has proved to be too slow to be analytically useful. Further work with cadmium will be carried out with sulphur analogues of β- diketones as these are known to form more stable complexes with cadmium than do the parent compounds.

Volatilisation of iron and chromium with β- diketones, on the other hand, has been more successful. Some observations of reactions of iron are given in Table 1. Ferric oxide may be confidently expected to react with HFA. The volatilisation reactions of iron and its compounds occur at convenient temperatures and evidently show sufficient temperature differences for speciation. Detailed radiotracer trials will be carried out to confirm this conclusion. A comprehensive study of the volatilisation reactions of β- diketones has now been undertaken.

The prospect of using a chemical reaction for deposition of metals from volatile complexes is attractive. The deposit would be easily manipulated for NAA. Further, if reaction temperatures are sufficiently lower than deposition temperatures, it should be possible to employ true chemical transport in a much simpler apparatus.

Auxiliary Studies:

When fully developed, the reactive gas extraction technique described above should permit multielement speciation analyses in which elements are distinguished by gamma-ray spectrometry during NAA. In preparation for this type of multielement analysis, research is also being conducted in NAA. These studies include methods for the analysis of gamma-ray spectra and computer aided design of procedures that use NAA.

A new approach to the estimation of the spectral baseline in gamma-ray spectra has been successfully developed (4) and compared to previous techniques. The new method compared favourably with older ones and permits greater flexibility in spectrum analysis.

Our research in automated method design is currently focussed on a new approach to the optimisation of conditions used in NAA. This method, called simplex optimisation by advance prediction (S.O.A.P.), uses spectra predicted (5) from the expected composition of the sample to provide values of a response function for use in simplex optimisation calculations (6,7). The adjustable parameters currently employed are irradiation, decay and counting times and sample size. Figure 4. shows the operation of the program.

The response function adopted for optimisation of single-element determinations is proportional to the detection limit (7) of the element of interest.

$$R= (2.71+4.65B^{0.5})/(A)$$

B   baseline area under peak
A   peak area

Figure 5. shows the response surface for the determination of arsenic in a hypothetical airborne particulate sample. The circle indicates the optimum found by the simplex program.

The simplex approach to optimisation of NAA has proven to be effective and very flexible. New response functions are easily implemented and limits to adjustable parameters and other quantities such as total count rate are easily achieved.

A comprehensive data set for airborne particulates is nearly complete and will be used to establish optimum conditions for the determination of important elements by NAA. A response function is being devised for the optimisation of multielement NAA and will be used to determine the conditions that permit the maximum information to be extracted from an airborne particulate sample.

References:

1. W.D. Ross and R.E. Sievers, Anal. Chem. $\underline{41}$ 1109 (1969)

2. R.L. VanHemert, L.B. Spendlove and R.E. Sievers, J. Electrochem. Soc. $\underline{112}$ 1123 (1965)

3. P.C. Uden and D.E. Henderson, Analyst $\underline{102}$ 889 (1977)

4. D.D. Burgess and R. Tervo, Nucl. Insts. Meth. $\underline{214}$ 431 (1983)

5. V.P. Guinn, E. Garzanov and E. Cortes, J. Radioanal. Chem. $\underline{43}$ 599 (1978)

6. J.A. Nelder and R. Mead, Comput. J. $\underline{7}$ 308 (1965)

7. R. O'Neill, Appl. Stat. $\underline{20}$ 338 (1971)

Table 1.   Volatilisation with TFA

Sample	Temperature (at start of deposition) $^\circ C$
Fe	200
$FeCl_3$	190
$FeCl_2 \cdot xH_2O$	180
Fe_2O_3	no reaction to 350
Cr	250
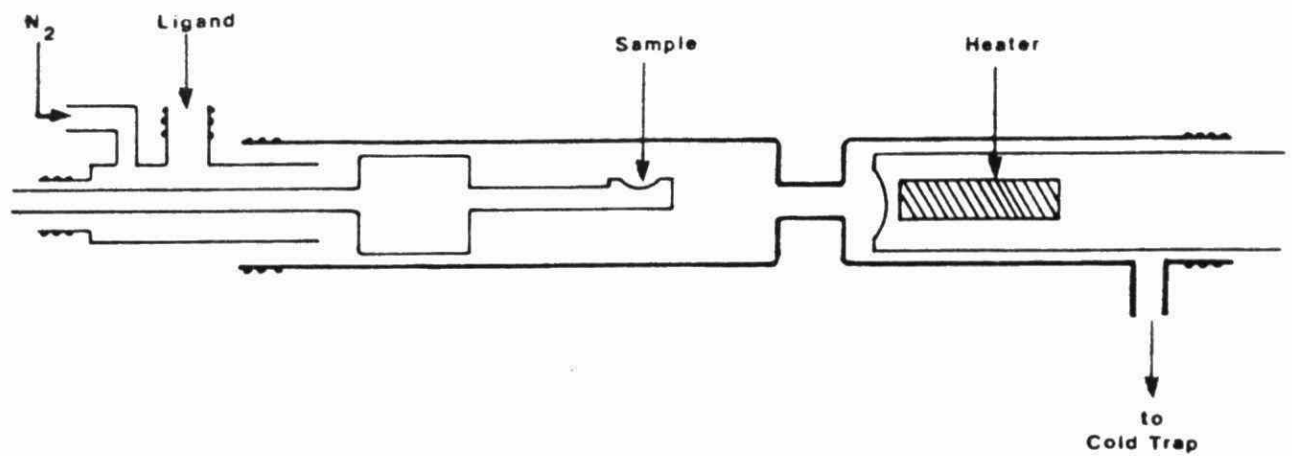
Figure 1. Volatilisation Apparatus
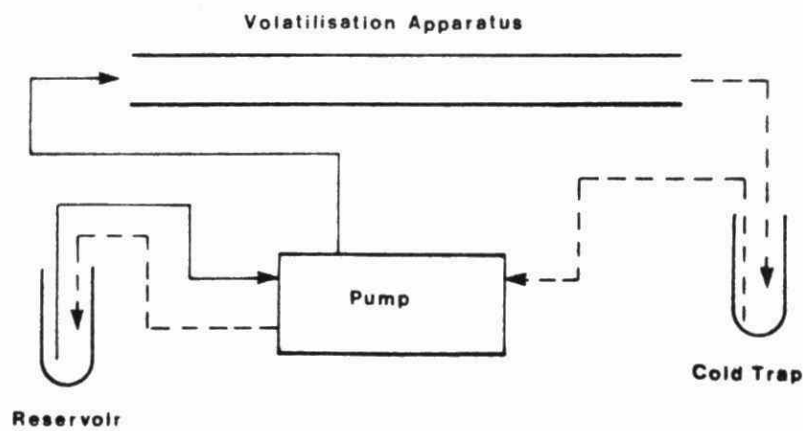
Figure 2. Ligand Recycling
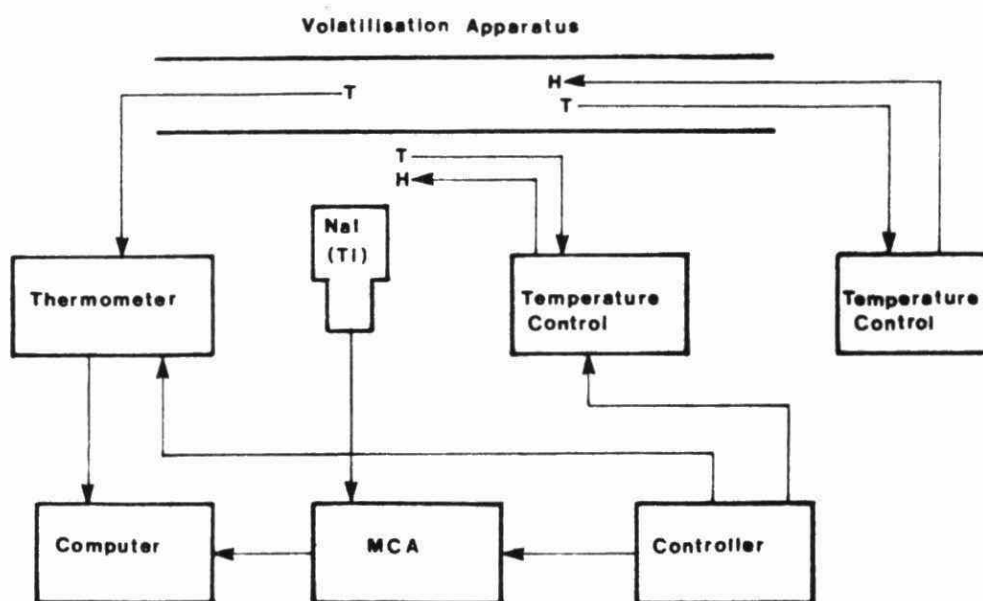
Figure 3. Experiment Control : H heater T thermocouple
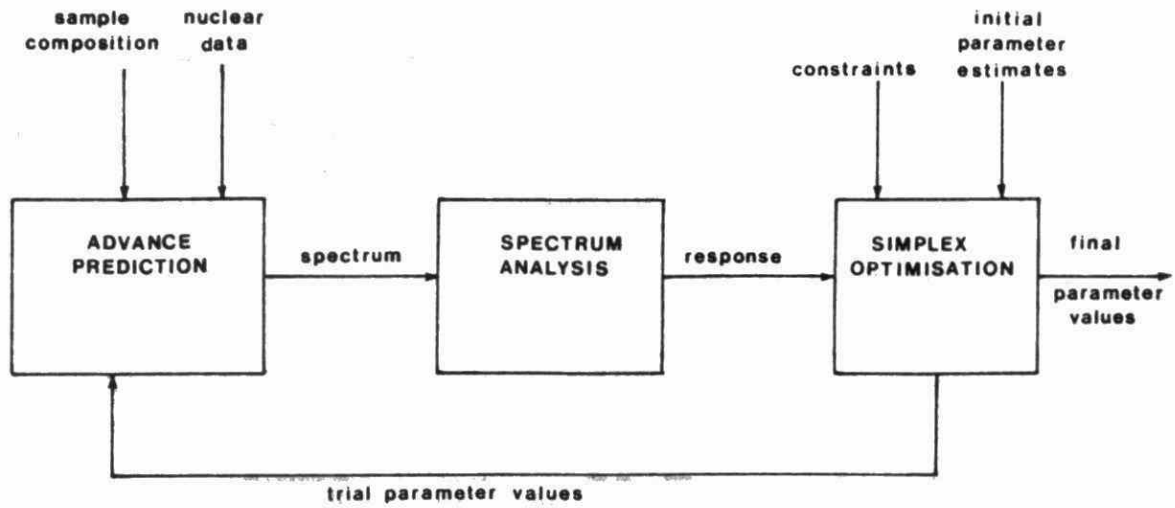
Figure 4. Simplex Optimisation by Advance Prediction
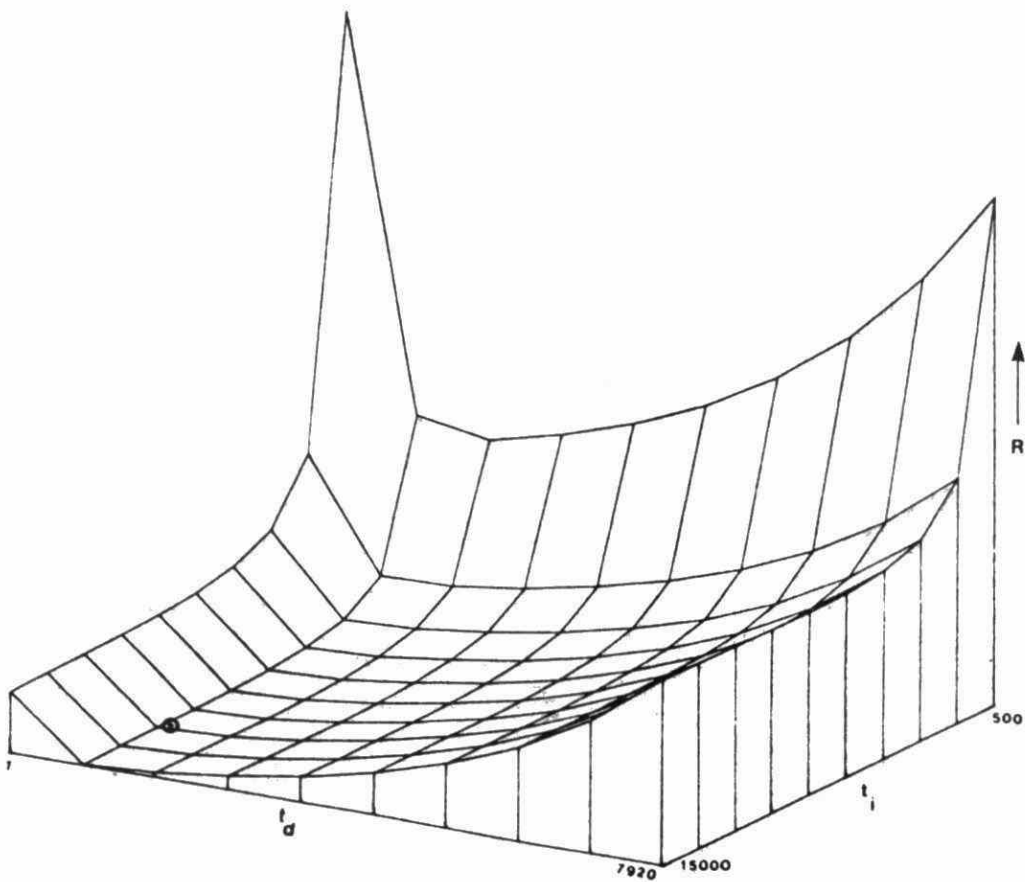
Figure 5. Response Surface for As in Airborne Particulates

Vapour Phase Photolysis of Chlorinated Aromatic Hydrocarbons

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Abstract

Although there have been almost no published accounts of the vapour phase photochemistry of chlorinated aromatic hydrocarbons, the subject has potential environmental significance because typical aryl chloride pollutants are somewhat volatile. They therefore are potentially subject to solar degradation in the atmosphere. Our research is directed to studying the modes of photodecomposition of typical aromatic pollutants (chlorinated benzenes and biphenyls) under inert (N_2) and reactive (O_2) atmospheres.

In this presentation, the literature on aryl halide photochemistry is reviewed; since most of the previous studies have used solution phase conditions, we have attempted to indicate the extent to which any of the observations may be relevant to gas phase photochemistry. Finally, we present a brief account of the experimental procedures we are using, together with some preliminary results on product identification in the chlorobenzene series.

Chlorinated aromatic hydrocarbons are widespread and persistent pollutants. The EPA priority pollutant list (1978) mentions chlorobenzene, the three dichlorobenzenes, 1,2,4-trichlorobenzenes, hexachlorobenzene, and the polychlorinated biphenyls (PCB's) as examples of priority pollutants. DDT is an example of a chlorobenzene derivative (Figure 1).

In addition to the many studies of the biological chemistry of these chlorinated hydrocarbons, there have also been numerous photochemical studies. The justification for these photochemical studies has been that because these chlorinated hydrocarbons absorb light in the region of the solar spectrum (although very inefficiently) there may be a chance that sunlight assisted degradation is one of the routes by which these substances disappear from the environment.

To date, almost all the laboratory studies of these substances have been made using solutions of the chlorinated hydrocarbons in organic or in partly aqueous solvents. Any environmental aspect to this work therefore concentrates on the prospect that sunlight may decompose these substances in solution, for example, in bodies of water. Recently photochemical degradation using artificial light was used to destroy a "real sample" of one of the related class of compounds, the chlorinated dibenzodioxins, at a chemical manufacturing plant in the United States[1].

Pollutants such as the PCB's may be found in locations very remote from

any industrial activity. They have, for example, been found in the snows of Antarctica[2]. It follows that they must have reached this location by transport through the atmosphere. This is reasonable, given the relatively high volatility of many of these compounds.

Sample calculations

1. Chlorobenzene, b.p. $131^\circ C$ = 404 K

   enthalpy of vaporization = 41.0 kJ mol^{-1}

   $$\ln P_2/P_1 = \frac{\Delta H}{R}\left(\frac{T_2-T_1}{T_1 T_2}\right)$$

   $P_{273K} = 2.9 \times 10^{-3}$ atm = 2.9×10^2 Pa

2. 2-Chlorobiphenyl, b.p. $274^\circ C$ = 547 K

   ΔH_{vap} estimated from Trouton's Rule = 48,000 J mol^{-1}

   $\ln (P_{273}/P_{547}) = -10.6$

   $P_{273K} = 2.4 \times 10^{-5}$ atm = 2.4 Pa

3. 4-Chlorobiphenyl, b.p. $317^\circ C$ = 590 K

   ΔH_{vap} estimated from Trouton's Rule = 52,000 J mol^{-1}

   $\ln (P_{273}/P_{590}) = -12.3$

   $P_{273K} = 4.6 \times 10^{-6}$ atm = 0.46 Pa

The research in which we are engaged is to understand the gas phase photochemistry of chlorinated aromatic compounds. We want to discover 1) the products of these reactions and 2) the detailed mechanisms by which the reactions occur so that 3) we can try to estimate whether the photolysis of these compounds by sunlight plays a significant role in their chemistry while they are present in the atmosphere. Because our research project commenced only on September 6, 1983, this account will be mainly devoted to what was already known about the photochemistry of these compounds.

Let us first make a digression concerning the concept of mechanism in a photochemical reaction. Besides the usual questions: "what intermediate species are formed en route to the final product?" and "how rapidly does each step in the pathway occur?", photochemical reactions pose two additional kinds of questions. These are: "which excited state of the starting material is involved?" and, "what is the 'quantum efficiency' of the reaction?" where the quantum efficiency is defined as the ratio below.

Quantum efficiency, ϕ = $\dfrac{\text{No. of molecules undergoing the process of interest}}{\text{Total no. of photons absorbed by the reactant}}$

The "Jablonski Diagram" (Figure 2) summarises the main processes of interest. Singlet excited states differ from triplets in the relative alignments of the electron spins; for our purposes here we note only that triplet excited states tend to be quite a lot longer lived (microseconds) than singlet excited states (nanoseconds). The processes available to the excited states are:

- return to the ground state with luminescence

- return to the ground state without luminescence

- singlet to triplet intersystem crossing (singlet only)

- chemical reaction.

Chemical reaction will proceed with more than negligible quantum yield only if the rates of the other competing processes are not too fast. Of course, in absolute terms, all chemical reactions of excited states must be very rapid, because of the speed of the competing processes which deactivate excited states. Consequently, photochemical reactions tend to occur only when they are energy releasing and have only small activation barriers.

Liquid Phase Studies

Chlorinated aromatic compounds have been investigated photochemically for 15-20 years. Essentially all these studies have been made in the liquid phase (usually in solution) and there is very little background of gas phase photochemistry upon which to draw. The primary photochemical act in most cases appears to be cleavage of the carbon-to-chlorine chemical bond (eq. 1); this is the weakest bond in the molecule.

e.g.   + $\cdot C\ell$ [1]
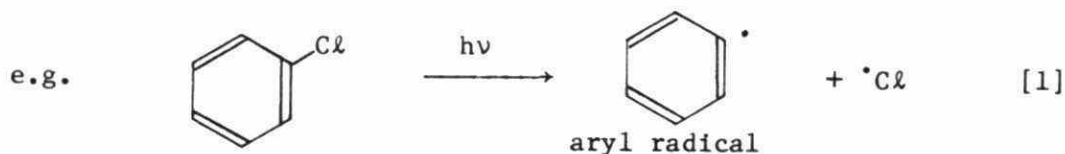
The products of this initial cleavage are free radicals, which are very reactive. In solution, the final products depend upon what other compounds are available to undergo reaction with these radicals (Table 1).

Table 1: Products from photolysis of chlorobenzene
in various solvents

Solvent	Structure	Product(s)	Structure
neat chlorobenzene[3]		chlorobiphenyls	
benzene[4]		biphenyl	
cyclohexane[5]		benzene hydrogen chloride	+ HCℓ

In benzene (or equivalently in neat liquid chlorobenzene) the favored reaction of the aryl radical is addition to the aromatic solvent, eventually giving a substitution product of the biphenyl series. Aliphatic solvents, exemplified by cyclohexane (but including alcohols, ethers, etc.) suffer

hydrogen abstraction, and the aryl radical is reduced to benzene.

The photochemical efficiency of the reaction is strongly dependent on the energetics of eq. [1][6]. The excited state involved is usually a triplet; if the energy of the state above the ground state is comparable with, or greater than, the C-Cℓ bond energy, dissociation occurs efficiently (high quantum yield). If the excited state energy is too low the reaction is very inefficient. Most estimates place the C-Cℓ bond energy in the region of 330 kJ mol^{-1}; Table 2 gives the triplet energies of some representative aryl chlorides.

Table 2. Triplet energies of aromatic systems

System		Triplet Energy, kJ mol^{-1}
Phenyl		350
Naphthyl		250
Biphenyl	not o-substituted	280
	o-substituted	330

Chlorinated benzenes and o-chlorinated biphenyls (Figure 3) tend to undergo dissociation with high efficiency ϕ 0.1-0.6. On the other hand, chlorinated napthalenes and non-o-chlorinated biphenyls are very resistant to photolysis, ϕ <10^{-3}. (For some of the chlorinated benzenes and naphthalenes, the reactions of photo-dimers complicate the picture, but these are not of concern at environmental concentrations). We may note also that aromatic bromides and, especially, iodides are more easily photodissociated than chlorides, because the bond to carbon is weaker.

A particular problem arises in the biphenyl series in that the o-chloro compounds are much more photolabile than those lacking o-substituents. This

is a steric effect. The ground state of biphenyl is best described as having a single bond between the two benzene rings (Figure 4a); a comparable description of the excited state has the rings coplanar (Figure 4b). Substitutents larger than hydrogen cause steric compression, which is relieved when the substituent (chlorine) departs. Additionally, the energy content of the o-excited state is higher, again because of steric compression (Table 2). This point is discussed in more detail in reference 7.

Vapour phase studies

Only one report of the gas phase photochemistry of chlorobenzene has appeared[8]. In that work, Ichimura and Mori photolysed chlorobenzene in an atmosphere of ethane. The products observed were benzene, HCl, and butane. It is clear that under these conditions the reaction is exactly analogous to that observed in solution with, for example, cyclohexane. The products are explained by Scheme 1.

Scheme 1

$$PhCl^* \longrightarrow Ph^{\cdot} + Cl^{\cdot}$$

$$Ph^{\cdot} + C_2H_6 \longrightarrow PhH + C_2H_5{}^{\cdot}$$

$$Cl^{\cdot} + C_2H_6 \longrightarrow HCl + C_2H_5{}^{\cdot}$$

$$2C_2H_5{}^{\cdot} \longrightarrow C_4H_{10}$$
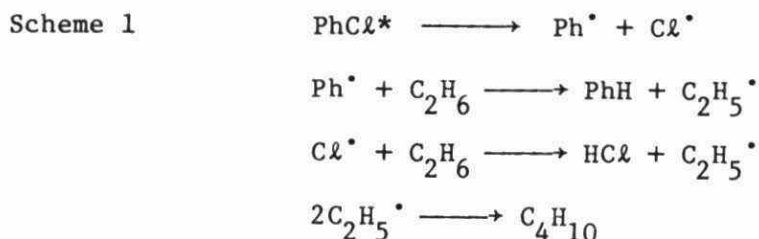
For the present purposes, what is significant is that the quantum yield of reaction (0.4) is very similar to that found in solution. In solution, there could be cage recombination (eq. 2) which would make the observed quantum yield of decomposition smaller than the primary quantum yield of decomposition.

$$(Cl^{\cdot} + Ph^{\cdot}) \longrightarrow PhCl \qquad [2]$$
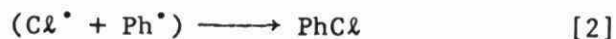
The similarity of quantum yields suggests that this is not a problem in practice; more importantly, it suggests a primary $\phi_{decomp.}$ of ca 0.4 should

be observed in any gas phase reaction of chlorobenzene. Therefore in ordinary air, we would expect that the first formed radicals in scheme 1 should disappear by reaction with oxygen, eq. 3.

e.g.
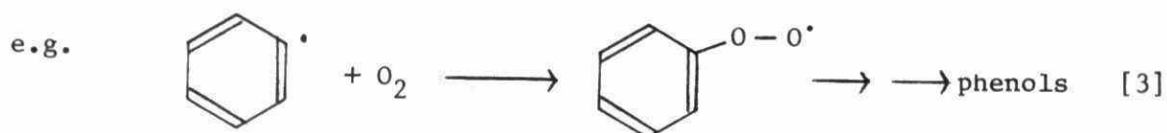
$$\longrightarrow \longrightarrow \text{phenols} \quad [3]$$

The expectation of eq. 3 appears to be fulfilled in the chlorobiphenyl series, where the only published gas phase study, that of Hustert and Korte[9], indeed gave a phenolic product eq. 4.

$$[4]$$
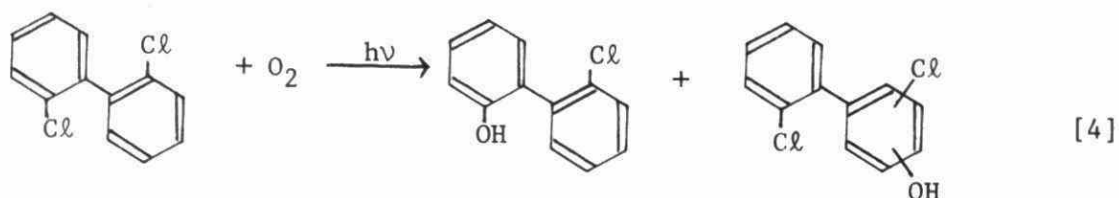
In this study, 2,2'-dichlorobiphenyl underwent photodecomposition to afford two products. In one of them, hydroxyl replaced one of the chlorine substituents, presumably by some reaction analogous to eq. 3. The other product was incompletely identified, but contained a hydroxyl function additional to the two chlorines. We would expect, again by analogy with the solution phase studies, that PCB's containing ortho chlorines should be much more photolabile in the gas phase than those without.

In further work, Prof. Korte and his students have concentrated extensively on the "photomineralization" of PCB's and other substances. Photomineralization is the term used by this research group for the complete photodegradation (to HCl and CO_2) of the pollutants adsorbed on particles of silica gel. Such reactions are intended to model the fate of the pollutant adsorbed on particulate matter in the atmosphere. They are thus

complementary to our studies in which the pollutants are photolysed in the gas phase.

Among chloro compounds which are not aromatic, a research group at Dow Chemical Co. has studied the simulated at atmospheric photodecomposition of simple aliphatic chlorides such as chlorinated ethenes and ethylenes[10]. These reactions were carried out with 10 ppm of the organic compound in the presence of ca. 5 ppm of NO to simulate a polluted atmosphere. Under conditions controlled to imitate bright sunlight, all the mono-, di-, and trichloroethylenes had half lives for decomposition $\tau_{1/2}$ of 5-12 h. Chlorobenzene is mentioned briefly in this paper, its $\tau_{1/2}$ was ca. 9 h. No products were identified.

The present work

The reaction chamber which we have had made consists of a 12 litre flask equipped with three ground glass joints. The largest of these (60/50) accommodates an inversion well apparatus which projects inside the flask. The lamp, a 450 W "Hanovia" medium pressure mercury arc, rests in the centre of the immersion well and is cooled by a flow of water. We have available both quartz and Pyrex inversion wells; the former transmits all radiation $\lambda > 220$ nm, the latter only $\lambda > 290$ nm. Although the Pyrex equipment more closely simulates sunlight, it is convenient to use the quartz for speedier reaction times in our exploratory experiments.

The remaining inlets to the flask are used to admit and remove gas. The chloroaromatic compound is placed in a reservoir, which may be heated if necessary. Gas (N_2 or O_2) is passed slowly through the reservoir, and entrains some of the vapour of the chlorocompound. After the gases leave the reaction chamber, they are condensed at -78°C in a dry ice cold trap. After reaction the contents of the trap and the reaction flask are

separately dissolved in a suitable solvent, and analysed by vapour phase

chromotography and GC-mass spectrometry.

Our first experiments have been carried out using chlorobenzene with

the quartz equipment and a nitrogen (inert) atmosphere. Under static

conditions (no flow, the chlorobenzene placed directly in the reaction

flask), most of the product is an intractable polymer. At low conversions

we see the expected products of bimolecular reactions e.g.

monochlorobiphenyls (compare Table 1). Under conditions of flow, at least

two unimolecular products (similar volatility to starting material) are

formed. These compounds appear to decompose thermally on standing and are

not yet identified. We speculate that they may be "valence isomers" of the

starting material. Similar valence isomers have been detected previously

upon photolysis of benzene and its methyl derivatives.

References

1. J.H. Exner (editor). "Detoxication of Hazardous Wastes" Ann Arbor
   Science, Ann Arbor, Michigan, 1982, Chapters 16 and 18.

2. R.W. Risebrough, W. Walker, T.T. Schmidt, B.W. de Lappe, and C.W.
   Connors, Nature, 264, 738 (1976).

3. J.A. Barltrop, N.J. Bunce, an A. Thomson, J. Chem. Soc. (c), 1142 (1967).

4. G.E. Robinson and J.M. Vernon, J. Chem. Soc. (c), 3363 (1971).

5. D.R. Arnold and P.C. Wong, J. Am. Chem. Soc., 99, 3361 (1977).

6. N.J. Bunce, M.D. Bergsma, J.P. Bergsma, W. De Graaf, Y. Kumar, and L.
   Ravanal, J. Org. Chem., 45, 3708 (1980).

7. N.J. Bunce, Chemosphere, 11, 701 (1982).

8. T. Ichimura and Y. Mori, J. Chem. Phys., 58, 288 (1973).

9. K. Hustert and F. Korte, Chemosphere, 4, 153 (1974).

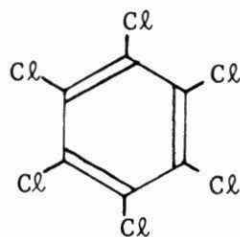10. W.L.Dilling, C.J. Bredeweg, and N.B. Tefertiller, Env. Sci. Technol.,
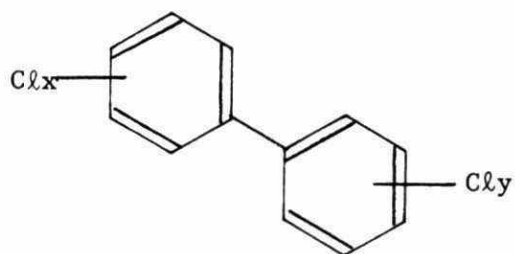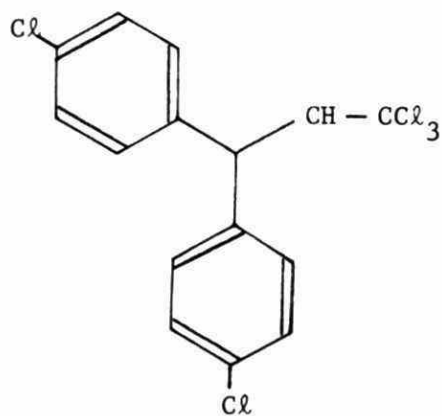   10, 351 (1976).
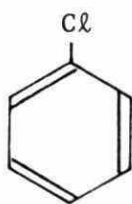
chlorobenzene
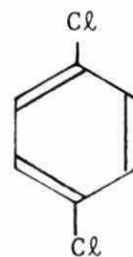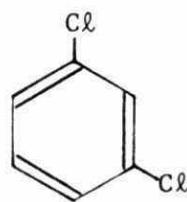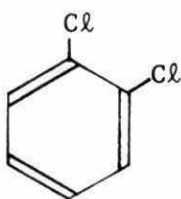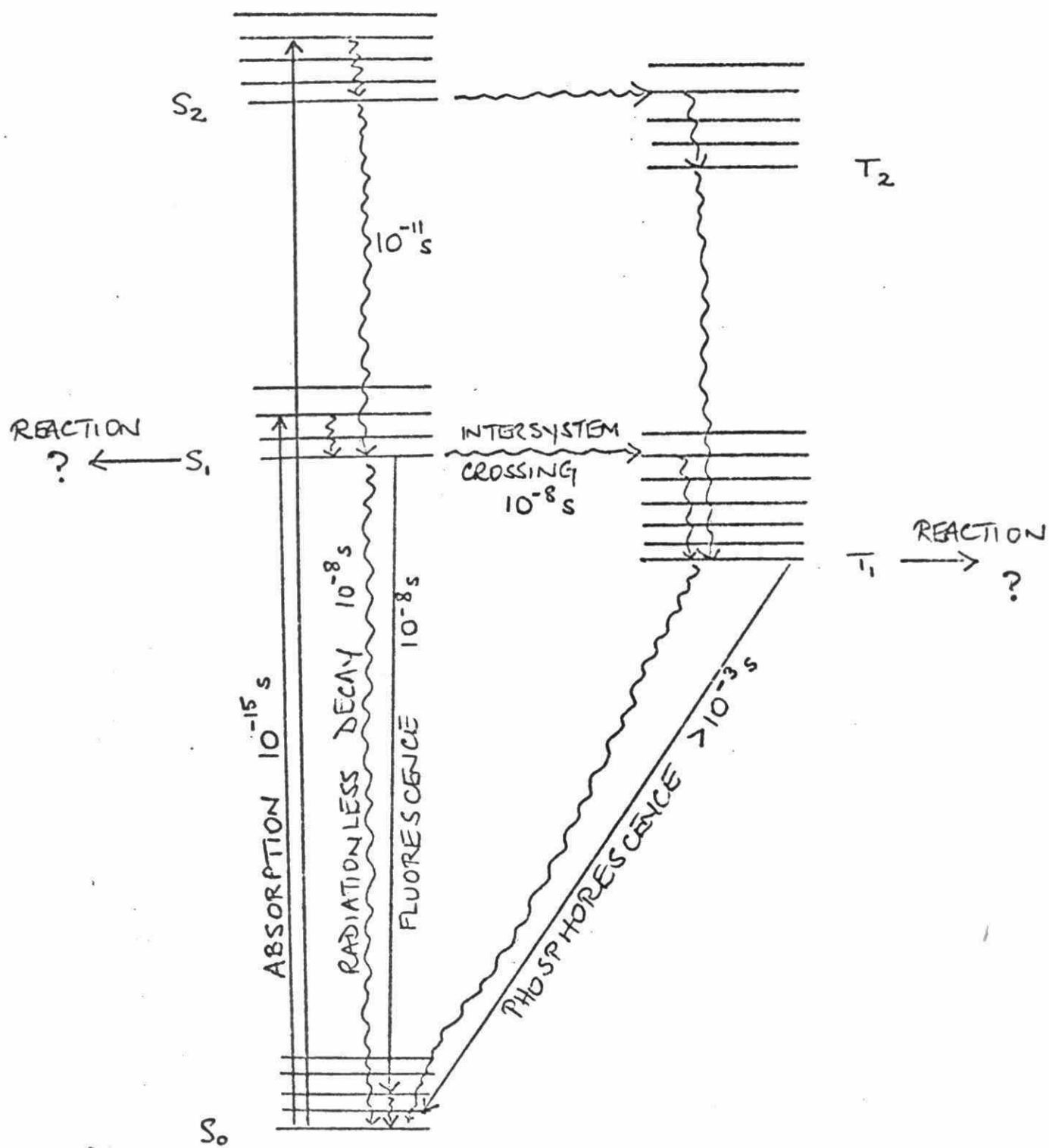
o- , m- , and p-dichlorobenzenes
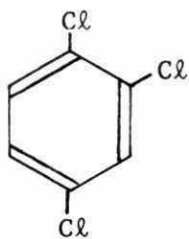
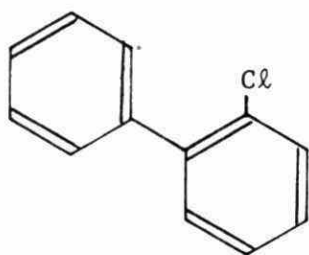1,2,4-trichlorobenzene
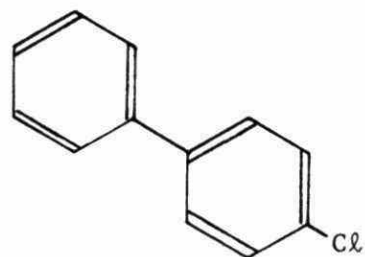
hexachlorobenzene

x and y in the range 1-5

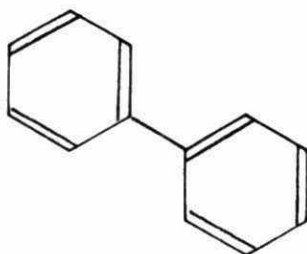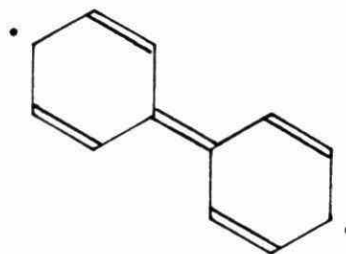p,p' — DDT

"JABLONSKI DIAGRAM"

o-chlorobiphenyl

p-chlorobiphenyl

Figure 3: Examples of o- and non-o-chlorobiphenyls

(a)

(b)

Figure 4: Valence bond representations of (a) ground state
(b) first excited state of biphenyl